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Better information about development

WHEN the United Nations Conference on Science and Technology for Development (UNCSTD) comes to an end in Vienna in late August, what will it leave behind for the future? Many hope it will not spawn another UN agency or office, as there are enough of them already. But in individual countries there could be a valuable legacy: as a result of preparations for UNCSTD many nations have brought together a wide range of people—administrators, politicians, industrialists, trade unionists, academics—to discuss national problems, be they those of rich or poor countries. If these networks survive and continue to talk to each other then UNCSTD will not have been in vain.

Britain has kept such a low profile both domestically and internationally over the UNCSTD meeting that to talk about networks having emerged from any national debate on the subject would be entirely fatuous. And yet there is at present the possibility of raising public awareness about development questions.

The Overseas Development Ministry recently commissioned a survey on public attitudes towards overseas development and found a substantial decline in support for British aid since the last such survey (in 1969).

Whilst only a few per cent are against richer countries giving help to poorer countries, 39% of those interviewed were against Britain herself giving aid. And few were able to give even an order of magnitude estimate of how much the country spends on overseas aid, generally overestimating the figure. As interviews progressed and those interviewed were told Britain's actual expenditure, a significant shift in

attitudes occurred and support for more expenditure grew substantially.

Part of the reason for ignorance and distrust of overseas aid could simply be low public awareness of what is involved in an aid programme. Such a conclusion would be reinforced by comparative statistics on how much Britain spends on education about development: Britain's spending on education about overseas development has until recently amounted to about £215,000 per annum—about 0.3p per capita. This contrasts very unfavourably with countries such as Germany and Canada (spending 2p and 7p per capita respectively), and doesn't bear comparison at all with expenditure of 10p per capita in the Netherlands or 15p in Sweden. Now spending is going up enormously; a recent ODM working party on the subject recommended a budget of £2.7 millions for development education by 1982 (*Overseas Development Paper 14*, HMSO) and the government has accepted these proposals.

Universities should take advantage of this new scheme. By no means all ignorance of the problems of the developing world is confined to those who never benefited from higher education; indeed most students are unlikely to be made to think at all about these matters during their formal coursework except, perhaps, in a handful of lectures in an optional course on science and society. Better informed graduates in science and technology might make for more soundly-based judgements later in life. The financial means for providing better information are now available. Who will take them up? □

JET—an insoluble salary problem

READERS of the classified advertisement section of *Nature*—most of our readers tell us they turn to it first—will not be unaware of wide differentials in salary. If you work in a British university, or for the British civil service, the salary scale will be tightly defined to the fourth significant figure of a very modest sum. Find a post in a continental university and the salary doubles, at the least. The cost of living goes up too, of course, although few would deny that, taken all in all, the scientist in England is ill-paid in comparison with his continental colleagues.

It is the posts in international organisations which really raise the eyebrows, however. Many of these positions are either tax-free or subject to very modest taxes indeed. Recently, for instance, readers will have noticed such choice offerings as the directorship of an internationally sponsored laboratory in Monaco, no less, at £24,000 per annum, tax free. This is hardly an unusual sum; researchers with several years' experience can command the same at CERN.

What happens, however, when internationally sponsored laboratories, with their traditionally high tax-free salaries come to Britain where the natives are ill-paid? The answer is—real recruitment problems, as those working on the Joint European Torus (JET) programme are finding out in their early days at Culham.

At the professional level, roughly one-third of the em-

ployees at JET are British, the rest are from other participating nations; at the senior technician level Britain supplies about 50% of the staff. The United Kingdom Atomic Energy Authority pays the salaries of Britons at its own standard rates subject, of course, to British taxes. Euratom supports the rest, who pay only a very modest 'community tax'.

Were Euratom to pay at the same rate as it does in Brussels, say, the differential between British and non-British employees' pay would be grotesque, so a figure of 62% of the Brussels rate has been established. Italians, for instance, still find this sort of salary attractive, but the same cannot be said for Germans—so there are differential recruitment problems. And even so, the 62% figure means that the net salaries of non-British employees are between 1.5 and 2 times greater than those of British counterparts. It is generally accepted that a factor of 1.3 to 1.5 is adequate compensation for living in a foreign country; there is little doubt that higher figures are ultimately going to be bad for morale.

At present, even if there are recruitment problems, the high level of motivation of the staff makes the salary question less important. But JET is a long-haul project, and the salary issue will not go away unless something quite remarkable happens to Britain and the pound sterling. □



'Emergency' ban on 2,4,5-T herbicide in US

Clive Cookson reports from Washington on swift reaction to a study linking 2,4,5-T with an increase in miscarriages

THE US Environmental Protection Agency has taken emergency action to ban the use of the controversial herbicide 2,4,5-T, after receiving new evidence linking it to a high incidence of miscarriages in Oregon.

"Studies completed only days ago show a high miscarriage rate immediately following the spraying of 2,4,5-T in the forests around Alsea, Oregon," explained EPA deputy administrator Barbara Blum. "This alarming correlation comes at a time when seven million pounds of 2,4,5-T are about to be used to control weeds on power line rights-of-way and in pastures, and to manage forests across the nation".

These uses will be stopped immediately by "emergency suspension"—the most drastic action EPA can take against a herbicide or pesticide, and the first time it has ever been invoked. The closely related herbicide, Silvex, which is used primarily to kill weeds on suburban lawns, is also covered by the order. But the remaining legal uses of 2,4,5-T, on open ranges and rice fields, are not included and may continue, the EPA said, "because they appear at this time not to involve human exposure comparable to the suspended uses".

However, the Environmental Defense Fund has rejected this argument and petitioned EPA for a total ban on all uses.

2,4,5-T [2,4,5-trichlorophenoxyacetic acid] and Silvex [2-(2,4,5-trichlorophenoxy)propionic acid] are both contaminated with small amounts of 'dioxin' (TCDD), which produces birth defects, miscarriages and tumours in animals, even at extremely low concentrations. The dioxin is thought to be responsible for their ill effects on animals.

For eight years, US environmentalists have been fighting legal and administrative battles against the continued use of 2,4,5-T (which was applied as a military defoliant in Vietnam and was widely blamed for the high incidence of birth defects there). But the EPA claims that its latest study is the first to link the herbicide clearly to ill effect on humans.

The agency commissioned the study last year when nine women from Alsea wrote to say they had had miscarriages after the national forest there was sprayed with 2,4,5-T. Scientists from Colorado State University and the University of Miami medical school compared miscarriages in the Alsea basin (western Oregon) with a control

population in rural eastern Oregon. They reported that:

- The miscarriage rate in the Alsea area was significantly higher than in the control area, where 2,4,5-T was not sprayed.

- The rate peaked dramatically in June in each of the six years studied, two or three months after the annual spring spraying. The spontaneous abortion index in June over the period 1972-77 was 130 per 1,000 births in Alsea and 46 per 1,000 in the control area.

Dr Blum said: "It's a remarkable correlation. While it is not proof of a cause and effect relationship, it is highly suggestive, particularly in light of animal data, and gives great cause for concern". She estimated that four million people across the United States would be protected by the emergency action.

The EPA claims that the short-term economic impact of the ban will be slight, because alternative herbicides are available for use on pasture and rights-of-way, and only 0.2% of the country's commercial forest area is sprayed annually. The long-term cost to forestry of a permanent ban could be \$7m-12m a year, because other herbicides are less effective and more expensive.

Dow Chemical is the leading manufacturer of 2,4,5-T and Silvex. A few

Dioxin: the 10-year battle that began with Agent Orange

Alastair Hay traces the steps leading up to the EPA ban

EVER since the late 1960s when the herbicide 2,4,5-T was found to contain the extremely toxic contaminant 2,3,7,8-tetrachlorodibenzodioxin (dioxin), there has been much concern over its continued use. It has already been banned in some European countries.

According to Mr Frank Parsons of the EPA's Pesticide Programme, 2,4,5-T is the biggest single issue the agency has had to deal with so far. In April last year, the agency issued its "Rebuttable Presumption against Registration and continued Registration of Pesticide Products containing 2,4,5-T", and interested parties were given 45 days in which to submit evidence supporting or opposing the use. An additional 60 days grace was granted following a request from one

of the major manufacturers for more time to present its case.

Parsons says that the EPA has received over 2,700 submissions on 2,4,5-T ranging from a 2,300-page memorandum from Dow Chemical—the major manufacturer of 2,4,5-T—supporting its use, to individuals claiming they were sprayed with the herbicide and their "hair dropped out". Asked how seriously the agency considered such comments, Parsons said that if enough of these claims are made "we can't ignore them." But the main protagonists in the debate are the chemical manufacturers and environmental pressure groups.

During the Vietnam war, Dow supplied most of the 2,4,5-T used by US forces in their defoliation programmes in Vietnam. It was used at that time in a 1:1 combination with 2,4 dichlorophenoxyacetic acid, called Agent Orange.

In 1969, Agent Orange was reported to be teratogenic (causing foetal abnormalities) in a study published by the US Bionetics Research Laboratory. Although it was subsequently shown that it was the dioxin con-

taminant—sometimes present in quantities in excess of 30 ppm—which was teratogenic and not 2,4,5-T itself, the Bionetics study led the US Government to withdraw Agent Orange from Vietnam and to restrict 2,4,5-T use in the US to forestry and clearing rights of way.

It was the high dioxin contamination which marked 2,4,5-T as a teratogen. The EPA reasoned, therefore, that reducing this contamination to less than 0.1 ppm might render it safe. However, as more evidence about dioxin's toxicity accrued, the agency was forced to review the situation. In 1972, Dow obtained a temporary court injunction preventing EPA from taking further action against 2,4,5-T. The following year, this was overturned by the US Court of Appeal. When the first hearings were held by the EPA in 1974 they proved inconclusive: there was insufficient data supporting allegations that dioxin in 2,4,5-T presented a health risk, and an order proposing the cancellation of 2,4,5-T was withdrawn.

The latest evaluation of the herbicide has thus been a continuation of

other firms also make them. Although the emergency suspension took effect from the moment it was announced on 1 March, the companies can appeal to have it lifted. When the suspension issue is settled, "cancellation hearings" will begin, to determine whether the herbicides should be banned permanently. That will take years to decide. □

EPA disputes Dow claim

The Environment Protection Agency has disputed claims by Dow Chemical that some dioxin compounds found in the environment are the result of normal combustion processes. The claim was made by the chemical company last November as part of its defence against charges that pollution associated with its herbicide production plant at Midland in Michigan was resulting in trace elements in fish in the Tittabawassee River.

Dow claimed that, since it had found dioxins associated with a number of combustion sources including car exhaust and charcoal grills, dioxins were formed by normal combustion processes. But EPA says this claim was based on purely circumstantial evidence. It points out that evidence submitted by Dow to the EPA shows dioxin levels two to four times higher in Midland than in other locations checked. It also criticises Dow for using analytical techniques and procedures which have not yet been corroborated by other scientists. □

the earlier reviews, but with the difference that all sides have been claiming sufficient evidence to support their case. Mr Gary Jones, a spokesman for Dow Chemical told *Nature* in an interview before the EPA ban was announced that the company's view was that "if, with all the confidence we have on the safety of 2,4,5-T, we can't say it is safe, then we can't prove the safety of aspirin".

The company felt that the attack on 2,4,5-T is part of a campaign by environmental groups whose campaign will not stop with the banning of the herbicide. Dow sees itself as defending an attack on the chemical industry by environmentalists who, Jones said, "want to go back to windmills". Jones added that Dow was defending 2,4,5-T not because the herbicide is a large revenue earner—sales worth \$12m represent only about 0.2% of the company's total annual income—but because it is "science which should be determining 2,4,5-T safety, not emotion".

The basis of Dow's argument is that dioxin at 0.1 ppm in 2,4,5-T presents no human health hazard. The company has been defending this

Dow admits 'poor' lab results

THE US Environmental Protection Agency (EPA) has not only been reviewing the safety of the herbicide 2,4,5-T; it is also engaged in a major collaborative programme with US scientists to compare laboratory measurements of 2,3,7,8-tetrachlorodibenzodioxin (dioxin) — a toxic contaminant present in the herbicide.

Central to the EPA's concern about dioxin is the poor reproducibility of dioxin measurements. In an attempt to find out which laboratories were reliable the EPA induced five labs to take part in a collaborative programme. The results of this collaboration were reviewed at a recent meeting in Washington organised by the agency.

The potential threat posed by 2,4,5-T is that its dioxin contaminant will enter the human food chain. US scientists claimed recently that dioxin had been detected in beef fat and human milk. The same two vehicles were therefore chosen by the EPA for its programme. Fat and milk samples were 'spiked' with dioxin in quantities ranging from 0–8 ppt (parts in 10^{12}) and extracts prepared suitable for analysis. The extracted samples, together with known standards—all prepared by the EPA Pesticide Monitoring Laboratory at Bay St Louis, Mississippi—were randomised and sent to the five participants: the Dow Chemical Company; Harvard University; the University of

Nebraska; Wright State University, Ohio; and the EPA Health Effects Research Laboratory (HERL) in North Carolina.

If proof were needed that there is inter-laboratory variation, then this study provided it. Only two laboratories performed well in the study—those of Dr Michael Gross of Nebraska and Dr Matthew Meselson of Harvard. The EPA's own laboratory results were regarded as reasonable by scientists present at the meeting but those of the other two were felt to be poor—Dow's being the less accurate.

The fact that Dow Chemical performed badly in this collaborative programme has not passed unnoticed by some scientists in the field. They argue that it should have been Dow, a company with a long-standing interest in dioxin measurements, which came out at the top of this league table. Dr Warren Crummett, of Dow, confessed that his results were "poor". "They were worse than normal", he told *Nature*. According to Crummett, some of the blame lies with the method of dioxin extraction chosen by the EPA. Had Dow done its own extracting procedure, then, Crummett was convinced, his laboratory's results would have been much better.

Of the five laboratories, only Gross's performed well in identifying 'false positives' and 'false negatives'. Discon-

claim not only before the EPA but also in a New York federal court. Dow and five other US manufacturers of 2,4,5-T are being sued for \$10m by the estate of a 28-year-old Vietnam veteran, Paul Reutershan, who died in December last year of terminal cancer of the colon.

Reutershan, a helicopter crew chief in the Vietnam war, flew on several herbicide spraying missions during which Agent Orange was used. Ten years later when he was diagnosed as having cancer, Reutershan was convinced that dioxin was the cause. Following his death, a new suit was brought on his behalf as well as on that of "all American servicemen whose health has been damaged, or is likely to be damaged, because of contact with Agent Orange". The new suit has been filed by a veteran attorney of chemical contamination cases, Victor Yannacome Jr, and is likely to prove a test case.

There is little comfort for the litigants, however, from the Veterans Administration (VA). The VA has reviewed some 500 claims from ex-servicemen—mainly from the Chicago area—that exposure to 2,4,5-T has

damaged their health. But, a spokesman said, the administration has not been able to prove that "any of these claims are herbicide-related".

Environmental groups such as the Environmental Defense Fund insist that the very presence of dioxin renders the use of 2,4,5-T dangerous. For these groups the fact that dioxin has been shown in three separate studies to be a carcinogen, and that it is always present as a contaminant in 2,4,5-T—albeit at a low concentration—is sufficient to proscribe its use in any circumstances.

Balancing these conflicting arguments was apparently a difficult exercise for the EPA. Before the ban was announced, the Agency admitted that it was practically impossible to do a "precise reckoning" of the risks and benefits of the herbicide. One official of the Agency confessed that the "political climate is terrific on this issue" and that it did complicate matters. However, as he saw it, the EPA's role was to review the evidence and if the Agency made both the environmentalists and industry unhappy then "we are probably doing our job".

certainly the other four all failed to single out enough of these 'false' values. There was some agreement among those present at the Washington meeting that dioxin values close to the detection limit—regarded as being 9 ppt—did complicate matters and that perhaps some false positives and negatives were unavoidable.

In future, the researchers will probably abide by the gentleman's agreement practised so far that only values which are at least 2½ times greater than the noise levels will be regarded as true measurements. This decision could well eliminate some of the false predictions. Some scientists—notably those at Dow—feel that dioxin estimations lower than 9 ppt must, therefore, still be treated with scepticism. Others still disagree, and argue that it depends on the methods of estimation.

Contamination of the dioxin extracts by other chlorinated hydrocarbons—PCBs, for example—makes interpre-

tation more difficult. Dow argue that this could be one reason for their poor results and that their extraction would produce a "cleaner" sample. However, contamination is not the only problem. There is still no measure of agreement between the practitioners on the methods to be used to estimate the recovery of the final dioxin sample.

Dioxin chlorinated with ³⁷Cl is used in most cases for this purpose: the tracer is normally incubated with samples before extraction, but there are still doubts in the minds of some of the researchers that this tracer 'equilibrates' with the natural dioxin to a sufficient extent so that estimates of the final recovery are indeed accurate.

There are doubts too about mass spectrometry readings and views differ as to whether two dioxin ions with a selected mass from natural dioxin (320 m/e, 322 m/e) should be measured and the results averaged or whether one mass reading is sufficient. The

results in the EPA review are more or less independent of the method of measurement—good and poor results were recorded by both mass spectrometry methods.

Although some of these views have still to be resolved there was still general agreement at the meeting that the EPA should publish the results of this collaborative programme as soon as possible and that it should be subject to peer review. However, whether the reviewers agree with the consensus of opinion expressed at the meeting that dioxin analysis has become far better in the past few years remains to be seen.

What they will undoubtedly say is that there ought to be even more collaboration between laboratories to ensure greater reproducibility of results. As for the EPA, it will probably be given credit for initiating the first major stage in this collaboration.

Alastair Hay

Chemicals in food: new US framework proposed

A NEW framework for regulating toxic substances in food, which would give greater discriminatory power to the Food and Drug Administration, and allow the benefits as well as the risks of a particular substance to be taken into account, has been suggested by a study group of the National Academy of Sciences.

The proposal is made in a report published last Friday by the Academy's Institute of Medicine and National Research Council, prepared at the request of Congress following the controversy two years ago over whether or not saccharin is carcinogenic, and if so what should be done about it.

According to the panel of scientists, lawyers and public policy experts which prepared the report, the proposed framework—under which a substance could be placed in a category of high, moderate or low risk, with discrimination given to the FDA over handling substances in each category—would effectively separate the scientific assessment of risk from its social consequence.

In a letter submitting the report to Health Secretary Mr Joseph Califano, for example, Dr Philip Handler, president of the academy, says: "estimation of risk is a scientific matter, albeit not always readily feasible. Decision concerning the acceptability and management of a given risk is an intrinsically political question to be returned to the polity for determination."

Not all of the panel, however, agreed that the distinction is a tenable one. A minority report, disputing the suggestion that toxic food substances can

be divided into three categories according to varying degrees of risk, says that there is "no scientifically defensible way" of doing this.

The statement, signed by five of the 37 members of the committees responsible for the report, says that "the ability of science to quantify human risk has not advanced sufficiently since the formulation of the Delaney Amendment [which banned all food additives shown to cause cancer in laboratory animals] to permit the construction of a scientific rationale for such a scheme."

The report forms half of a two-part study carried out by the NAS under the terms of an act passed by Congress in 1977, which placed an 18-month moratorium on a ban on saccharin following the disclosure that it had been found carcinogenic in laboratory animals. The first part of the report was published last November, and concluded that saccharin was indeed a potential human carcinogen, although its potency was probably low compared to other known cancer-causing agents, such as cigarettes.

The second report is concerned with the public policy implications of this and similar findings for the regulation of food safety. It recommends a single policy applicable to all foodstuffs, additives and contaminants, and says that regulatory agencies should be able to do more than simply ban or not ban a particular substance.

"This report suggests that a realistic policy would be to weigh the estimated level of risk of a substance in our food supply against the perceived benefits of its use, and to employ informed

judgment as a basis for regulatory decisions," according to Dr David A. Hamburg, president of the Institute of Medicine.

A major problem presented by current law, the report says, is that most cases of both health and non-health benefits are excluded from regulatory consideration. "It is better to have benefits defined, evaluated and openly considered when that is possible", it says.

Speaking in Washington last week Professor Don K. Price, one of the panel members responsible for the report, denied that by shifting the focus of decision-making from Congress to a regulatory agency, the process would become more vulnerable to outside pressures. "The committee felt that the present scheme, which in theory asks the FDA to make a purely scientific decision, has not worked very well, and in many cases political and other considerations have been smuggled in—or, as in the saccharin case, have given rise to other problems", he said.

"This new system would require regulatory agencies to get a statement on the scientific aspects from a research institution, and the subsequent decision-making process can then be made clear, public and open, so that you can deal with it better than if you ignored outside pressure".

The committee made no particular recommendation for the marketing of saccharin, and the minority statement disagreed with the view of the majority that "a total immediate ban of saccharin would not be a sound regulatory step at the present time."

David Dickson

2,000 US scientists boycott USSR

IN what one participant called a "grass-roots" protest at the treatment of dissidents by the Soviet authorities, over 1,800 US scientists have signed a pledge to "withhold all personal co-operation with the Soviet Union" until Yuri Orlov and Anatoly Shcharansky, the two USSR scientists gaoled last summer, are released.

A further 600 US scientists have signed a separate pledge that they will not attend international conferences in the Soviet Union, will collaborate with and attend lectures by Soviet visitors only if they are invited, will oppose any enlargement of scientific and technical exchange programmes between the USSR and the US, and will "campaign against the transfer of sophisticated Western technology to the Soviet Union."

Signatures on the two petitions have been gathered by the Berkeley-based organisation Scientists for Orlov and Shcharansky, set up last summer when the trials of the two dissidents was taking place. SOS, as the organisation is known, says that its policy is to take concrete actions that deprive the USSR of some of the benefits of American science and technology, particularly in areas such as computing where the US has a considerable lead.

"The actions taken by SOS are a completely spontaneous response to Soviet policy, and are not connected to any position or policy of the US Government," Professor Kurt Gottfried, professor of physics at Cornell University, said last week.

"Our opposition to Soviet oppression is a limited response to Soviet actions, not a search for confrontation. Despite our commitment to human rights we view any linkage between human rights and the effort to achieve arms control as irresponsible," Professor Gottfried said, adding that SOS fully supported President Carter's efforts to negotiate a second Strategic Arms Limitation Treaty.

Gottfried was echoing Sakharov, who recently declared that human rights should not be made a *sine qua non* of the signing of the SALT agreement. While supporting Sakharov's opinion, John Macdonald, Q.C., Yuri Orlov's London-based "defence lawyer" nevertheless feels that the signing of SALT could lead to the release of political prisoners such as Orlov and Shcharanskii. It could well provide an opportunity for President Carter to press home the human rights issue—and for Mr Brezhnev to make a gesture which the Soviet Union could afford.

David Dickson and Vera Rich

Yugoslavia hits nuclear snag

PROPOSALS to build Yugoslavia's second nuclear power station on the island of Vir (see map) are meeting with opposition from the island's inhabitants, as well as the municipal authorities and 'socio-political organisations' of the nearby resort town of Zadar on the central Adriatic coast.

The local inhabitants and authorities seem more interested in preserving their amenities as a tourist area than in the alleged advantages which the plant would bring to the area—which is notably short in energy.

The vigorous opposition to the nuclear power station comes only a few weeks after a series of major power cuts so severe that even long-standing atheists were observed buying votive candles from church-porch stalls. Yugoslavia's major power source is hydroelectricity, and the dry autumn and early winter led in December to cuts of up to eight hours a day.

It was just such a drought, six years ago, that led to a big drive for alternative sources of energy. Five foreign enterprises competed for the contract for the first Yugoslav power station. Westinghouse won, and work began at Krsko, near the Slovenia-Croatia border.

The target date for completion was January 1979, but construction was consistently behind schedule.

The delays, however, did not relegate



nuclear power in the minds of the Yugoslav planners. By April 1978, the power industry was talking in terms of 12 nuclear power stations with a total annual capacity of 66,000,000 MWh by the end of the century.

So far, however, the Krsko station is still not operational and the siting of the second is in doubt. The final decision on the Vir site has not yet been reached—but local opposition suggests that a better choice might be Ivanicgrad, which not only has the necessary water resources, but is also a major industrial centre, where the local population may be less likely to raise environmental issues.

Vera Rich

UK must support talent, says minister

MRS SHIRLEY WILLIAMS, UK secretary of State for Education and Science, is concerned that not enough young scientists are finding jobs—because most available posts are filled by people in their thirties and forties who are unlikely to move on. "If science in Britain isn't to sink gently into the second class", she told the Association of British Science Writers earlier this week, "then we will have to try to make room for each generation of outstanding talent."

Making more money available to the research councils should help solve the problem, she said, and that was one of the reasons behind the 4% increase in the science budget announced at the end of last year. Another reason for increasing the science vote was to take the pressure off the SRC, whose budget had been "blocked up by existing commitments" and to encourage the funding of interesting or important work by the other research councils.

A white paper is to be published this week, said Mrs. Williams, about the 'customer-contractor' principle whereby a portion of research council funds is administered by ministries, which

commission research relevant to their needs. For the system to work, says Mrs Williams, "commissioned research has to be handled on a basis of stability and on a relatively long lead time". Problems occur if the level of research commissioned by a ministry is suddenly changed.

There is also a limit to that level and, according to Mrs Williams, the Agricultural Research Council's budget has already reached it. The increase in the science vote, however, should relieve some of the research council's dependence on commissioned research.

Mrs Williams is also concerned that the UK does not make the most of its innovative ideas. "It is sad", she says, "that one so often sees the brilliant idea worked on in a university laboratory being patented in another country and then sold back to us." She would like to see small companies encouraged to take up new ideas and the establishment of a "small enterprise board" which would assume for enterprise a similar role to that taken by the National Research Development Corporation for technology.

Judy Redfearn

news in brief

Reductions urged in medical radiation exposures: The elimination of all unnecessary medical and dental exposure to radiation was "critically important" to protect the health of the public, US Secretary for Health, Education and Welfare, Mr Joseph A. Califano, said last week.

Exposures resulting from X-rays, radiation therapy and nuclear medicine accounted for almost 90% of all the man-made radiation to which the general population was exposed, Mr Califano said, adding that he had ordered efforts by the DHEW through the Food and Drug Administration to reduce such exposures to be speeded up.

Commenting on Mr Califano's order, Dr John C. Villforth, director of the US Government's Bureau of Radiological Health, said that medical radiation should be cut to half of present levels.

"Approximately 30% of all medical exposures are unnecessary," said Dr Villforth, adding that many were done for non-medical reasons, such as protecting doctors from malpractice suits. The radiation dosage used in "necessary" medical procedures could be cut by another 30%, using the most recent X-ray films and machines.

However Mr Califano said that the US government was not yet prepared to recommend a reduction in permissible occupational exposure levels which has recently been suggested by a number of US scientists following growing evidence of the potential dangers of low levels of radiation previously treated as safe.

"We do not believe that the science is adequate to justify further reductions" he said, although stressing that more scientific research was needed to settle the issue, and that the problem was a "major public health issue."

Mr Califano released draft working papers which have been prepared by an Interagency Task Force on the Health Effects of Ionising Radiation, set up by President Carter last year. Reviewing past and current research, the task force states that "existing knowledge is insufficient to provide an unequivocal answer to the low-dose question", and adds that a definitive answer may never be found.



"I see Peterson's having his influence!"

Head of OTA resigns to head wildlife society: Mr Russell Peterson, former governor of Delaware, has resigned as director of the US Congress' Office of Technology Assessment to become president of the National Audubon Society. Mr Peterson's departure after only 13 months of a six-year appointment is a major blow to an agency which has been plagued with political problems. Under his leadership it had appeared to be entering a period of relative stability and productive activity.

Nuclear safety issue heats up: For ten years Dr Stephen Hanauer, assistant director and senior technical safety official of the US Nuclear Regulatory Commission has kept a 'nugget' file on reactor safety incidents. The Union of Concerned Scientists obtained a copy of the file under the Freedom of Information Act and has published a 95-page summary. Among its contents are tales of safety parts installed upside down, thickly painted and varnished rods or pumps sticking, frozen pipes, clogged drains, faulty

soldering, radioactive cleaning dust, accidentally bumped switches causing overflows and a \$45m fire that firefighters were afraid to put out because of the danger of spreading plutonium throughout the area. The US Atomic Energy Commission commented that "the absence of more serious effects is largely the result of luck".

In the UK, Friends of the Earth is pressing the Secretary of State for Energy to fulfill his promise to release the complete report of the Health and Safety Executive on the safety of pressurised water reactors. Only a summary was released on 29 July 1977. FOE cites the scandal surrounding the Rasmussen Report in the US which was issued in summary form and has been subsequently found so unreliable that the Nuclear Regulatory Commission dissociated itself from it. The Rasmussen summary included predictions that an accident at the Enrico Fermi reactor on 6 October, 1966, when part of a core fused, should have happened only once in a million years, and the same techniques predicted that the accident on 5 June 1970 when the 600 megawatt Dresden 2 reactor near Chicago went out of control and discharged radioactive iodine into the environment should have occurred only once in 10^{12} years. FOE stresses that "there is a clear cut need for public access to the Health and Safety Executive's detailed reactor studies".

INFCE halfway through: The International Nuclear Fuel Cycle Evaluation Programme established at the Western nations summit meeting in London in 1977 to study the hazard of nuclear proliferation is now halfway through its life with a report to be issued "sometime in 1980". INFCE began with some acrimony between the US and the UK over the danger of proliferation from nuclear reprocessing. The UK government is committed to reprocessing while American opinion is opposed to it. This difference in outlook colours the discussions of the twelve working parties. The US tries to win support for an extensive system of intermediate spent fuel storage deposits while the UK argues that such a system will be unnecessary if there is extensive reprocessing. Other preliminary studies indicate that there is no preferred "proliferation resistant" design: all proposed nuclear fuel cycles are equally hazardous. Factors included in the analyses are: extent of reprocessing, number of plutonium storage sites, and physical organisation of the fuel cycle (where fuel is to be stored in relation to the reactor), how buildings are to be laid out and the number of people to be involved. These "technical" issues are proving to be relatively unimportant compared to the political problem of proliferation.

Wave power cost estimates cut: Wave power designers at Wavepower Ltd in Southampton have found improvements in the design and manufacture of the Cockerell raft (30 November 1978, page 433) that may reduce projected costs from 26p per kwh to 6p per kwh. The original Cockerell raft was built in three sections with two hinges. The centre section contained a costly and complex pumping system and gearing train needed to drive the turbine. A new single hinge design eliminates the centre section and replaces the pumping system with a simple large-vaned pump that will be part of the raft structure. A more sophisticated but standard turbine will be used to effect power take-off. "We have thrown away a lot of steel and mechanical engineering" says Jim Platts, Senior Project Engineer at Wavepower. The new design will cut costs from 26p to 12p per kwh and a further reduction to 6p can be achieved by mass production methods based on techniques used in oil pipeline manufacture. Additional savings must be found, however,

Let us stop regulating DNA research

By J. D. Watson

The Royal Society critique (*Nature*, 15 February 1979, page 510) of the proposed new approach by Britain's Genetic Manipulation Advisory Group (*Nature*, 9 November 1978, page 102) to recombinant DNA regulation is correct on at least one point. We do not have the facts on hand to make the quantitative risk assessments to decide whether a proposed experiment should be done under maximal, minimal, or no precautions.

Assigning numbers in the manner suggested by Dr Sydney Brenner implies that we have some real measure of prospective risk. However, to my knowledge there is no evidence that any prospective recombinant DNA experiment poses any realistic threat to any scientist who uses the tools of his trade, much less to society itself. I thus do not see any logical way to arrive at such numerical risks, and the whole GMAG exercise strikes me as having no more validity than quantitative religion.

To be sure some experiments like putting *E. coli* genes back into *E. coli* are thought safe by virtually all informed individuals. In contrast, the insertion of the gene for the botulinus toxin into *E. coli* might strike some competent scientists as risky. But how much additional peril will be generated beyond that involved with working with botulinus organism itself is not obvious.

The *a priori* guesses that different knowledgeable minds will give may at best tell us something about their respective neuroses or lack of them. Each of us has different thresholds of fear, be it rational or irrational. For my part, I already see so many unambiguous hazards, like dioxin, that I remain incapable of adding still one more unless I see some real numbers. Recombinant-DNA-induced diseases to me fall in the category of UFOs or witches. Others may take

them seriously, but they should not expect me to join in. I would not spend a penny trying to see if they exist and would regard as pure folly the decision to spend sizable sums of money on such projects.

I am thus appalled by the recent proposals of the Honourable Joseph Califano and Shirley Williams that their respective governmental departments (HEW and the Department of Education and Science) initiate extensive experiments to probe the presumptive safety of recombinant DNA experiments. The variety of prospective experiments is so legion that we have no possibility of checking even a tiny fraction for their potential danger.

'Recombinant-DNA-induced diseases, to me, fall in the category of UFOs or witches'

Moreover, the answers we could obtain would have no real carry-over to other even closely related situations. So we would be spending vast sums of money (Shirley Williams has proposed tens of million pounds) merely to give the facade of responsibility. In fact, we would be irresponsible by diverting money away from projects which unambiguously might advance science, if not society itself.

We must thus be careful not to undertake risk assessment experiments merely because they can be done. Here I part company with the Royal Society's position that we should see, for example, whether non-pathogenic strains of *E. coli* can be converted into pathogens through random insertion of foreign DNA. Such data are very unlikely to affect the way we work with recombinant DNA. Unless we directly take DNA

from pathogenic bacteria, the chances of a positive answer are low. But even if we were to generate a new pathogen, it is not obvious that it would pose any special risk.

Almost totally lacking in the past six years of seemingly never-ending conjectures about the dangers from recombinant DNA experimentation is recognition of the fact that the world is already filled with large varieties of pathogenic microorganisms of many types. Moreover, they are constantly mutating to give us new forms that we have not seen before. Some are constantly making us ill, and sometimes even doing us in. For the most part, however, we can fight them at least to a draw, and, of necessity, most of us do not spend much time worrying whether the next time they will get us. Otherwise we would be completely paralysed from any constructive behaviour.

Instead of continuing to waste masses of paper and the time of countless individuals who have real jobs to carry out, I believe we should quickly and resolutely abandon any form of recombinant DNA regulation. Concurrently our national leaders should announce that they will help push DNA research as fast as our national and corporate treasuries can permit.

At the same time we should, of course, remain alert to the rare possibility that one or more recombinant DNA research workers will come down with a disease we have not seen before. If that happens we naturally would have a story that deserves serious journalism, and for the first time there would be real facts for the risk assessor. But until that situation arises, and I'm doubtful that it ever will, we must make clear to the public that there is no more reason to fear recombinant DNA than there is to panic about the Loch Ness Monster. □

if wave power is to be competitive with the estimated 1p-2p per kwh cost of windpower and with the standard fossil fuel costs of 1½p per kwh.

American Institute of Physics wins tax exemption: The American Institute of Physics, the umbrella organisation for nine separate physics societies, has won its appeal against a tax ruling that would have deprived it of tax exempt status.

Many of the institute's activities, including the publication of a number of journals, had been placed in jeopardy by a decision taken last year by the Manhattan District Office of the Internal Revenue Service that it was not entitled to

tax exemption as a scientific, educational and charitable organisation.

The institute filed a protest in December against this ruling, which is thought to have been made on the grounds that the institution provides a specialist service to its member societies which is not available to the general public.

In a letter received by the institute last week, the national office of the IRS said that it had considered the institute's appeal, and had decided in its favour. "The entire physics community will be pleased with the Internal Revenue Service ruling", said Professor Philip Morse, chairman of the AIP's governing board.

'Fortunately for us, plants are very adaptable and exist in great diversity . . . they could thus continue indefinitely to supply us with renewable quantities of food, fibre, fuel and chemicals'

Professor D. O. Hall of King's College, London University, on biological solar energy conversion for fuels

BIOLOGICAL solar energy conversion via the process of photosynthesis produces every year stored energy in the form of biomass which is about ten times the world's annual use of energy. The amount of proven fossil fuel reserves below the earth is only equal to the present standing biomass (mostly trees) on the earth's surface while the total fossil fuel resources are probably only ten times this amount (Table 1). This massive-scale capture of solar energy and conversion into a stored product occurs with only a low overall efficiency of about 0.1% on a world-wide basis but because of the adaptability of plants it takes place and can be used over most of the earth.

It is not widely appreciated that one-sixth of the world's annual fuel supplies are wood fuel and that about half of all the trees cut down are used for cooking and heating. In the non-OPEC developing countries, which contain over 40% of the world's population, non-commercial fuel often comprises up to 90% of their total energy use. This non-commercial fuel includes wood, dung and agricultural waste and because of its nature is seldom thoroughly considered. Total wood fuel consumption is probably three times that usually shown in statistics, and about half of the world's population relies mainly on wood for their cooking (four-fifths of total household energy use) and heating. Furthermore, supply statistics of non-commercial energy can be out by factors of 10 or even 100.

The so-called 'second energy crisis' has arisen as a result of the diminishing supplies of non-commercial or traditional fuels such as wood, dung and straw. This has led to increasing deforestation, damage to ecosystems and rural poverty. Fuels-from-biomass programmes are now being considered in many developed and developing countries of the world but the biggest difficulty with implementing them seems to be the simplicity of the idea—the solution is too simple for such a complex problem!

In the past, photosynthesis has given us coal, oil and gas, fuelwood, food,

fibre and chemicals. The relative use of these fixed carbon sources has varied over the years and will undoubtedly do so in the future. However, now with abundant oil, the products of present-day photosynthesis are mainly evident to the developed world as food. We should re-examine and if possible, re-employ the previous systems; but, with today's increased population and standard of living, we cannot revert to old technology and must instead develop new means of using present-day photosynthetic systems more efficiently.

Each year plant photosynthesis fixes about 2×10^{11} tonnes of carbon with an energy content of 3×10^{21} J; this is about 10 times the world's annual energy use and 200 times our food-energy consumption. The efficiency on land may be about 0.2–0.3% overall, whereas average agriculture may be about 0.5% efficient. These efficiencies represent stored energy and not just the initial conversion efficiencies so often quoted in other energy systems.

All the atmospheric CO_2 is cycled through plants every 300 years, all the O_2 every 2,000 years and all the H_2O every 2 million years. The magnitude and role of photosynthesis is largely unrecognised principally because we use such a small fraction of the fixed carbon and don't realise the important recycling phenomena—any interference in this latter role from pollution could have serious consequences. The increasing content of CO_2 in the atmosphere (a 25% increase over the past 125 years) has become a worrying problem. However, there is no doubt that renewable biological (and photochemical) systems for fuel production will not contribute to the atmospheric CO_2 concentration and biomass may just help alleviate some of the problems by acting as a temporary carbon sink.

Fortunately for us, plants are very adaptable and exist in great diversity—they could thus continue indefinitely to supply us with renewable quantities of food, fibre, fuel and chemicals. If the impending liquid fuel problem which is predicted within the next 10 to 15 years comes about, we may turn to

plant products sooner than we expect. Let us be prepared!

Solar/biological systems could be realised to varying degrees over the short and long term. Some, such as the use of wood, biological and agricultural wastes, and energy farming, could be put into practice immediately, whereas others may never become practicable. Photobiological systems can be tailored to suit an individual country taking into consideration total available energy, local food and fibre production, ecological aspects, climate and land use. In all cases the total energy input (other than sunlight) into any biological system should be compared with the energy output and also with the energy consumed in the construction and operation of any other competing energy producing system.

Biomass

Solar energy is a very attractive source of energy for the future but it does have disadvantages. It is diffuse and intermittent on a daily and seasonal basis, thus collection and storage costs can be high. However plants are designed to capture diffuse radiation and store it for future use. Thus there is much serious thought (and money) being given to ideas of using biomass as a source of energy—especially for liquid fuels, but also for power generation. I am aware of biomass programmes in the UK, Ireland, France, Germany, Denmark, Sweden, USA, Canada, Mexico, Brazil, Australia, New Zealand, India, Philippines, Thailand, Israel and China.

The advantages and easily identified



problems with biomass systems are summarised in Table 2; the long term advantages are however very considerable. The programmes vary in their emphasis, and show that most of the research and development should be done locally. Such R & D is an ideal opportunity to encourage local scientists, engineers and administrators in one field of energy supply. Even if biomass systems do not become significant suppliers of energy in a specific country in the future, the spin off in terms of benefits to agriculture, forestry, land use patterns and bioconversion technology are, I think, significant.

In Table 3 I have attempted to summarise biomass products and their costs to give an idea how close some technologies are to economic reality. One must be fully aware of the assumptions involved before extrapolating or drawing firm conclusions from these figures.

USA

The USA has a very large R & D programme on biomass which had a 1977/1978 budget of \$22m and a proposed budget of \$42m for 1979. Burwell in an article called "Solar biomass energy: an overview of US potential" gives a good general picture and presents some of his own ideas of the best systems for the future. The 1975 US use of energy was 71×10^{18} J; the total standing forest inventory however has an energy content three times this annual energy use. The total annual biomass growth of commercial forests is 9.3×10^{18} J of which 6.6×10^{18} J is potentially collectible. Cropland agriculture produces energy totalling about 12×10^{18} J annually of which about 40% is represented by residues left on the land. Grain crops alone produce about 7.1×10^{18} J annually of which 5.9×10^{18} J is the net collectible energy yield, 3.8×10^{18} J of this being in the form of residues. A detailed analysis of "potentially usable biomass residues" shows that of the residues currently collected, 2.1×10^{18} J could be obtained from urban solid wastes, 1.0×10^{18} J from animal feedlots, canneries, wood manufacture, and so on. Uncollected residues such as cereal straw, corn stalks and logging residues could contribute 5×10^{18} J per annum. Burwell considers that major opportunities for energy provision lie in the use of forest residues and improved management of productive forest land. He also makes the interesting point that 60% of US cropland is dedicated to the production of livestock—and this excludes the contribution that 282 million hectares (38% of mainland US area) of pasture and rangeland make to livestock support. There are thus large areas of land which could be used in the future

for the production of biomass.

The concept of intensive silviculture biomass farms or energy farms has been one subject of detailed analysis. Fast-growing deciduous trees which coppice (resprout from stumps) have been examined, as have numerous other trees including those which fix nitrogen to ammonia. The use of agricultural residues as a source of energy in the US has been the subject of another major evaluation. A national total of 430 million dry tons is available each year, made up of 280m tons of crop residues, 120m tons of residues from logging and 30m tons from animal manure. (Excluded from these figures were residues from food processing, grazing animals, and hay and forage crops.) Theoretically this residue could supply 31% of the electricity, 20% of the natural gas, or 8% of the oil requirements of the US. This is very unlikely ever to happen since at

'If the impending liquid fuel problem comes about, we may turn to plant products sooner than we expect . . . Let us be prepared'

present 20% of the residues are used and 58% are returned to the soil, leaving only 22% classified as excess. However, these percentages will change as other uses (energy, chemicals, and so on) and prices emerge. At present electricity production from residues is cost-competitive at five study areas, process steam at seven areas and synthetic natural gas (compared to propane costs) at two areas.

Another US study says that adaptive systems should be encouraged which will "modify themselves to meet evolving needs and constraints". Such adaptive systems contrast with energy farms and the use of agriculture residues—but of course, the three systems can blend into each other depending on circumstances. Thus biomass intermediates (besides food) are processed into fuels or materials depending on relative price levels. Two examples cited are corn and sugarcane production. It is "theoretically possible to reorganise the present US corn biomass system to permit the production of 10 to 18×10^9 l of ethanol (or its equivalent in other fermentation products) while obtaining the same quantity of end-use food products in the form of beef, poultry and pork". The ethanol could be blended at 10% with a $\frac{1}{4}$ of the US gasoline. Sugar cane produces numerous byproducts such as molasses, alcohol and bagasse which can be used

for power generation, fermentable substrates and substitute wood. The study concludes that "fuels from biomass may possibly supply 10% or more of a curtailed US energy consumption" and that "knowing when and where to switch from emphasis on food and materials to emphasis on fuels is just as important as knowing how to produce the fuels."

Canada

Canadian studies on the large scale production of methanol from biomass show that by 2025 between 4 and 42% (depending on total energy use) of the transport fuels could be provided by such methanol. Methanol represents a rather unique fuel combining the portability of liquid petroleum products and the clean, even-burning characteristics of natural gas. The Federal Government recently announced a \$180m programme on biomass R & D and also provided incentives to industry to use wood and forest residues to provide energy.

Australia

In Australia, *Eucalyptus*, cassava (*Manihot*), *Hibiscus*, napier grass (*Pennisetum*) and sugarcane have been selected as potentially the most desirable high-yielding crops which can be harvested over the whole year. It was shown that alcohol produced from cassava (starch-rich) is an economically viable system, but that if processing to destroy cell walls is required the costs become too high. The cost of alcohol from cassava is calculated to be Australian \$250/tonne from a 100,000 tonne/year batch-process plant which compares favourably with the current market price of alcohol (Australian \$275/tonne) as an industrial solvent. Recent studies show that over half Australia's liquid fuel requirements could be produced from specifically-grown crops and agricultural residues.

New Zealand

With its very efficient agriculture, low population density and the fact that it uses a large proportion of its foreign exchange for petroleum purchases it is natural that New Zealand is considering biomass as a source of fuels. Current work shows that fodderbeet for ethanol and wood for methanol may be economic sources of liquid fuels.

Philippines

A feasibility study has shown that a 9,100 hectare fuel wood plantation "would supply the needs of a 75 MW steam power station if it were not more than 50 km distant". The investment requirements and cost of power produced looks favourable and competitive with oil-fired power stations of similar capacity. The best species of fast growing tree seems to be the "giant ipil-ipil" (*Leucaena leucocephala*) which fixes



Fodder beet in New Zealand: ideal for fermentation to alcohol.

nitrogen to ammonia.

Europe

In Europe a number of countries are conducting feasibility studies of the potential which biomass may have for supplying a source of energy and fuels in the future. Trial plantings of alder, willows, poplars, etc are being undertaken in addition to assessing energy yields from agricultural residues, urban wastes, techniques of conversion, waste land and forest potentials and algal systems.

Little has been published as yet but a recent study (Project Alter) in France by "Le Groupe de Bellevue" proposes that in the long term France could produce liquid and solid fuels, comprising 11% and 14% of its total energy requirement respectively, from biomass sources. Land use constraints will be a problem but considering Europe as a whole, its past vegetation history, with its diverse climates and land use patterns, and its already burgeoning food (including liquid) surpluses there may be far greater potential for biomass production than is commonly imagined.

Brazil

By far the most ambitious biomass programme which has been planned is that in Brazil for the production of alcohol from sugar cane, sorghum, cassava and other crops. This National Alcohol Programme (PNA or Proalcool) was established in November 1975. The alcohol will be used to blend with petrol—a mixture of up to 20% (by volume) requires no adjustment to the engine (over the past 10 years the State of Sao Paulo with over 1.3 million cars has varied the alcohol content of its petrol from 0.4 to 13.5%—and 18% in 1978—depending on the availability of alcohol and price of molasses). Up to August 1977, 141 new alcohol distilleries were authorised by Proalcool which would require an investment of about \$900m and would supply 3.2×10^9 litres of alcohol by 1980, about a fifth of the projected gasoline requirement. By 1985 total production of alcohol could reach 5 to 10×10^9 litres with a total investment of about \$3.15 billion. An economic

analysis of the production of alcohol from sugar cane and cassava calculated selling prices as fuel, ex-distillery, of \$0.33/l, 81% of the present retail price of gasoline on a volume basis. Brazil is clearly embarking on an ambitious programme of fuel import substitution using the natural advantages of land and climate which it has—and it may be a very useful demonstration to other countries. The indirect benefits such as saving foreign exchange, creating new employment, encouraging domestic technology and industry, and reducing pollution, are great.

Sahel region

The problem of deforestation and desertification has highlighted the lack of fuelwood in most countries of this region. It is a scarcity which is reaching crisis proportions in large parts of South Asia, the Sahel, Andean countries, Central America and the Caribbean.

The per capita requirement for cooking alone is about 0.5 m³ of wood/year. Total fuelwood requirement is probably about 1 ton which is equivalent to about 400 kg coal per person per year—equivalent of a 3 inch lump of coal per person per day. Demand from urban areas and industry to use char-

coal instead of the more expensive kerosene and oil is diverting woodfuel from rural areas, resulting in increasing forest and scrub destruction (often long distances from villages) and use of dung as a fuel instead of as a fertiliser. A recent Dutch study of the Sahel region points out two possible solutions: decreasing fuelwood demand by using stoves which reduce consumption by 70%, and increasing the supply of fuelwood by establishing "forest plantations". Costs of reforestation and fuelwood production have been calculated for the tree species suggested and the conclusion is that "under the conditions assumed it is an economically feasible activity". Naturally there are institutional problems which impinge on agriculture and other practices of the society but if such countries are to achieve even a modicum of internal fuel production they should seriously consider such biomass systems and set up fuelwood plantations as soon as possible.

India

A study in Tamil Nadu, South India, on the possibility of growing Casuarina (a nitrogen fixing tree) as a source of fuel for a power plant generating 100 MW electric has shown favourable

Table 1 Fossil fuel reserves and resources, biomass production and CO₂ balances

Proven reserves						Tonnes coal equivalent	
Coal	..	..	..	..	..	5×10^{11}	
Oil	..	..	..	..	..	2×10^{11}	
Gas	..	..	..	..	..	1×10^{11}	
						$8 \times 10^{11} \text{t}$	$= 25 \times 10^{21} \text{J}$
Estimated resources						Tonnes coal equivalent	
Coal	..	..	..	..	..	85×10^{11}	
Oil	..	..	..	..	..	5×10^{11}	
Gas	..	..	..	..	..	3×10^{11}	
Unconventional gas and oil	..	..	..	..	..	20×10^{11}	
						$113 \times 10^{11} \text{t}$	$= 300 \times 10^{21} \text{J}$
Fossil fuels used so far (1976)						$2 \times 10^{11} \text{t carbon}$	$= 6 \times 10^{21} \text{J}$
World's annual energy use							$3 \times 10^{20} \text{J}$
							$(5 \times 10^9 \text{t carbon from fossil fuels})$
Annual photosynthesis							
(a) net primary production	..	..	..	..	..	$8 \times 10^{10} \text{t carbon}$ $(2 \times 10^{11} \text{t organic matter})$	$= 3 \times 10^{21} \text{J}$
(b) cultivated land only	..	..	..	..	..	$0.4 \times 10^{10} \text{t carbon}$	
Stored in biomass							
(a) total (90% in trees)	..	..	..	..	..	$8 \times 10^{11} \text{t carbon}$	$= 20 \times 10^{21} \text{J}$
(b) cultivated land only (standing mass)	..	..	..	..	..	$0.06 \times 10^{11} \text{t carbon}$	
Atmospheric CO ₂	..	..	..	..	..	$7 \times 10^{11} \text{t carbon}$	
CO ₂ in ocean surface layers	..	..	..	..	..	$6 \times 10^{11} \text{t carbon}$	
Soil organic matter	..	..	..	..	..	$10-30 \times 10^{11} \text{t carbon}$	
Ocean organic matter	..	..	..	..	..	$17 \times 10^{11} \text{t carbon}$	

These data, although imprecise, show that (a) the world's annual use of energy is only 1/10 the annual photosynthetic energy storage, (b) stored biomass on the Earth's surface at present is equivalent to the proven fossil fuel reserves, (c) the total energy stored as fossil carbon only represents about 100 years of net photosynthesis, and (d) the amount of carbon stored in biomass is approximately the same as the atmospheric carbon (CO₂) and the carbon as CO₂ in the ocean surface layers.

economic and social benefits. In direct competition with coal a payback period for such an energy plantation would be from 15 to 30 years. An equivalent of 110 hectares is required to generate 1 MW electricity; about 11,000 labourers would be required, and the overall energy output/input ratios are high.

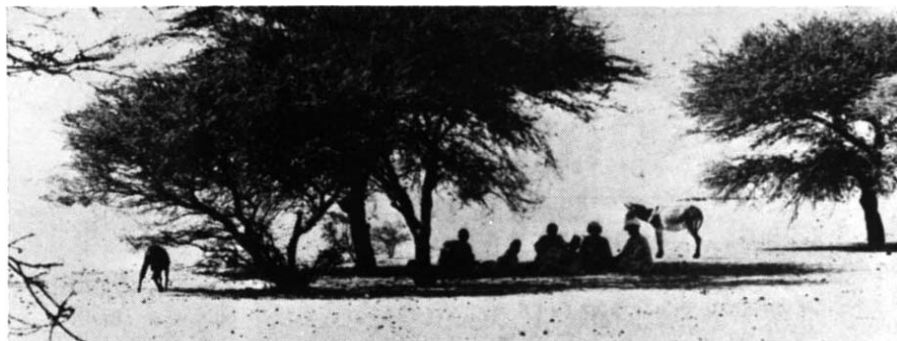
China

During the 1970s biogas plants have been perfected and installed at a rapid rate. It is estimated that there are now (1977) 4.7 million in operation and the methane so produced is used for cooking, crop drying, power generation and various other purposes. In Szechwan province alone, 17 million people use biogas for cooking and lighting; in some areas 80% of all rural households are served by biogas.

Algal systems

The idea of using algae and bacteria in biological solar energy systems is not new but has received more serious attention over the past few years. One advantage of such microbial systems is that they can be technologically sophisticated or simple depending on local conditions.

Many liquid and semi-solid wastes from houses, industries and farms are ideal for the growth of photosynthetic algae and bacteria, and under good conditions rapid growth with about 3–5% solar conversion efficiency can be obtained. The harvested algae may be fed directly to animals, fermented to produce methane, or burnt to produce electricity. Simultaneously, waste can be disposed of and water purified; it is estimated that such algal systems are half to three-quarters as expensive as conventional waste disposal systems in California. The harvested biomass can be fermented to methane, while the residues would contain virtually all the nitrogen and phosphorus of the algal biomass, so providing a good agricultural fertiliser. One acre of algal



The Sahel: scarcity of firewood has reached crisis proportions.

ponds could supply the fertiliser required by 10 to 50 acres of agriculture.

Future photosynthesis

One of the 'problems' with photosynthesis is that it requires a whole plant (or alga) to function—and the problem with whole plant photosynthesis is that its efficiency is usually low (less than 1%) since many limiting factors of the environment and the plant itself interact to determine the final overall efficiency. Thus a task for photosynthesis of the far future is to try to select and/or manipulate plants which will give higher yields with acceptable energy output/input ratios. We need much more effort placed on studies of whole plant physiology and biochemistry and their interactions with external (environmental) factors. Already this type of research is being increasingly funded by both industrial and government organisations.

Examples of the type of research which is being done or needs to be done are: photosynthetic mechanisms of carbon fixation; factors in bioproduction; genetic engineering using plant cell tissue cultures; plant selection and breeding to overcome stresses (drought, temperature or salinity); selection of plants and algae with useful products such as oil, glycerol, waxes, or pigments; nitrogen fixation and metabolism and its regulation by

photosynthesis.

Synthetic photosynthetic systems and photochemistry

Since whole plant photosynthesis operates under the burden of so many limiting factors (internal and external) would it be possible to construct artificial systems which mimic certain parts of the photosynthetic process and so produce useful products at higher efficiencies of solar energy conversion? (A 13% maximum efficiency of solar energy conversion is considered a practical limit to produce a storable product). I think that this is definitely feasible from a technical point of view but it will take some time to discover whether it could ever be economic. Plants in fact perform at least two unique reactions upon which all life depends and which have not yet been emulated in artificial (synthetic) systems: the splitting of water by visible light to produce oxygen and protons (hydrogen) and the fixation of CO_2 into organic compounds.

Photosynthesis is the key process in the living world and will continue to be so for the continuation of life as we know it. The development of photobiological energy conversion systems has long-term implications. We might well have an alternative way of providing ourselves with food, fuel fibre and chemicals in the next century.

Suggested timetable

for recommendations

● *Next 10 years*—fuels from residues, trees and existing crops; more efficient use of existing biofuels; demonstrations and training.

● *10–20 years*—increased residue and complete crop utilization, local energy crops and plantations in use.

● *After 20 years*—energy farming; improved plant species; artificial photobiology and photochemistry. □

Table 2 Biomass systems

Advantages	Problems
1. stores energy for use at will	1. land use competition
2. renewable	2. land areas required
3. dependent on technology already available, with minimum capital input	3. supply uncertainty in initial phases
4. can be developed with present manpower and material resources	4. fertilizer, soil and water requirements
5. reasonably priced	
6. ecologically inoffensive, and safe	
7. does not increase atmospheric CO_2	

Table 3 Predicted biomass energy products costs compared to conventional—USA (1981 \$)

Product	Cost from biomass (\$/10 ⁶ BTU)	Conventional cost (\$/10 ⁶ BTU)	Biomass Conventional
Methanol	8.4–15.9	8.4	1.0–1.9
Ethanol	15.0–36.3	19.6	0.8–1.9
Medium BTU gas ..	4.7– 7.4	3.0–5.0	0.9–2.5
Substitute natural gas	4.8– 7.3	3.0–5.0	1.0–2.4
Ammonia	5.8–11.4	7.4	0.8–1.5
Fuel oil	3.6– 7.9	3.2	1.1–2.5
Electricity	0.03–0.14(\$/KWH)	0.03–0.06(\$/KWH)	0.5–4.5

This article formed part of a study done by the author for a UNESCO study on "Fundamental World Energy Problems" and a UNEP project on "Photosynthesis in Relation to Bioproduction". References are not included but the author can supply details—also refer to article in "Solar Energy" (1979).

correspondence

Jobs gloom: a military solution?

SIR,—The problems of job opportunities and job security in science are serious (21/28 December, page 743). But solutions which may be highly desirable for individuals may be disastrous for science itself. While there are many exceptions, for most scientists the decline in research productivity between the years of 35 and 45 is unequivocal. Unfortunately this decline is only rarely matched by a compensating increase in teaching or administrative skills.

Although it may be anathema to most scientists to look to the military for an answer, I suggest that in this area it has much to teach us. Anyone becoming an army officer accepts that the career grade is Major and that, depending on the country concerned, those who are not promoted above this grade will retire between 40 and 45. Each level of promotion buys a few extra years until Field Marshals are appointed for life. A similar system in science would provide a long term solution to the present ills. It would work as follows.

Far more junior posts than are at present available would be created but these would automatically terminate at age 40. A gratuity equal to three years salary or a modest pension would then be provided. Retirement could be taken as early as 35 when a one year gratuity would be given.

Promotion above the basic career grade would depend on a realistic assessment of research, teaching and administrative contributions. Each promotion rung would buy an extra five years. Perhaps half the entry would be eliminated at the first hurdle and another half at the second, with relatively little attrition thereafter. The few highly productive scientists who remain active into old age would become 'Field Marshals' and would not be expected to retire.

The advantages of this system are many. As in the army, people would enter science knowing that unless they did very well their career would end at 40. This would eliminate the unenthusiastic at the beginning and provide a notable stimulus to research. Many junior posts would be available and the system would not be wrecked by a preponderance of individuals making little or no contribution. Institutions would not be reluctant to make early appointments for fear of having to maintain a person's employment until 65 or 70. In most cases scientists would know by the age of 35 whether or not they were going to be successful and could then begin to plan second careers in the comfortable knowledge that they would have three years in which to make adjustments. As with the military, organisations would develop which specialised in placing scientists in appropriate positions in industry or government. Industry, government, and hence science would all benefit from this influx of people with a genuine scientific background and not simply a first degree in science.

I do not underestimate the anguish

such a system would cause in the transition period. But some such radical approach is essential if we are not to replace the current problems of individuals by the much more serious one of an almost totally stagnant scientific community.

Yours faithfully,

DAVID F. HORROBIN

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No rabies virus at Glasgow

SIR,—I should be grateful for the opportunity of correcting an error which has appeared in your journal (11 January, page 81). This was a statement in which the Department of Virology at the University of Glasgow was cited as a laboratory holding a Category A Pathogen, namely rabies, as at 12 December 1978. This, I am happy to tell you, is not the case, as the small stock of rabies virus held by that department was destroyed in June 1977. It had, in fact, never been used for active research, and had remained in deep freeze for eight years before being destroyed.

I imagine that your source of information for this article was the draft of the Shooter report. I am informed by the Scottish Home and Health Department that they anticipate being able to update and correct this situation before the report is finally published.

Your faithfully,

ANDREW D. RANKINE

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Reassess hazards of recombinant DNA

SIR,—The comments (15 February, page 509) by a committee of the Royal Society on the recent published statement of the Genetic Manipulation Advisory Group will be widely welcomed and supported. However, I should like to comment on one of the suggestions they make.

It is argued that there is an urgent need for more research to evaluate the risks associated with the ingestion of organisms containing recombinant DNA. Before devoting yet more resources to the general hypothesis that recombinant DNA synthesised *in vitro* poses novel hazards to man, is there not a prior need to reconsider in which areas of recombinant DNA research there remain sound arguments indicating that the hypothesis should be taken seriously? The committee has properly criticised the Genetic Manipulation Advisory Group for publishing what are often called hazard scenarios without adequate argument or evidence supported by citation of relevant literature. But could not a similar criticism be made with equal validity against the hazards hypothesis in its entirety? The report of the working party chaired by Lord Ashby which was set up to evaluate this hypothesis made a number of assertions in favour of it but none of them was documented in the way the Royal Society committee advocates. Admittedly this report was written when responses to the possibilities suggested in

the Berg letter were more emotional than rational. All the more reason, now that four years have passed, for a cool reassessment of the questions before we embark on experimentation.

The most urgent need in 1979 surely is for a re-examination of the hazards hypothesis to determine which, if any, aspects of it remain credible enough to deserve further attention on scientific grounds. This reassessment can only begin if those who remain convinced of hazards are prepared to state their case in published form with the rigour urged by the Royal Society committee, and in a manner that permits rebuttal like any other scientific argument.

If such a reassessment suggests that areas of significant risk remain then let us devote resources to quantifying the risks in these areas. If, on the other hand, this reassessment suggests that the hypothesis is unfounded or if no one is prepared to argue the case to the contrary, then should not those biologists who have collectively exploited their authority to open the so-called debate on the hazards of genetic engineering be urged to use their authority to close it in like manner. The danger in what the Royal Society proposes is that it could lend further weight to a hypothesis that may not deserve it.

Yours faithfully,

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Energy forecasts mislead

SIR,—Your discussion of energy forecasts (18 January, page 162) misses the point. It is easy to forecast a low consumption of energy, and all too easy to make this forecast come true. The price of nuclear powered electricity has risen partially because of delays caused by those predicting low consumption of energy; as a result, demand has fallen. In a recent visit to England I noted that the head of a small college lived in a house I rejected as unsuitable as a research lecturer in 1952. This contraction in housing standards, and with it a contraction in demand for fuel, has reached other parts of society in the past few years.

The challenge is *not* to predict a low energy consumption and make it come true; but to work toward a just society, where the well being and convenience we have often correlated with energy consumption can be achieved with minimum financial, ecological and environmental costs. None of these costs have a one-to-one relationship with energy production or consumption, and to argue otherwise may be counter-productive. Those energy producers who concentrate solely on meeting "demand" have led us astray. Those who, like Mr Leach, are constantly trying to tell us that we *don't* need as much are equally guilty of keeping our concentration in the wrong place.

Yours faithfully,

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news and views

It is only five years since the first reports that lymphoid cells from unimmunised mice were able to kill certain lymphoid target cell lines (Herberman *et al. J. natn. Cancer Inst.* **51**, 1509; 1973). Since then, despite a deluge of papers dealing with the phenomenon, both in rodents and in man, little solid evidence has emerged to convince the cynical immunologist that this spontaneous or natural killing has the three attributes of immunological respectability—specificity of effect, specificity of immunisation, and specific memory. Moreover, the popular term, 'natural' killing only serves now to remind us how little is really understood of the role in nature of this function so readily studied *in vitro*.

Early results suggested that natural killer (NK) effector cells in the mouse lacked surface markers of both thymus-derived (T), and bone-marrow-derived (B) lymphocytes, and were neither phagocytic nor adherent to plastic. This null cell appeared to lyse preferentially lymphoid tumour cells expressing antigens coded by RNA tumour viruses (retraviruses), leading to the suggestion that NK cells were an *in vivo* surveillance mechanism against retravirus-induced tumours. For tumour immunologists this was an attractive hypothesis as it provided a substitute for the discredited T cell as a surveillance mechanism.

As is so often the case, unfortunately, later data have complicated the issue. No firm answers have yet emerged as to the nature and origin of the NK cell, the genetic control of its activity, or its specificity of killing, activation or memory. Nevertheless, recent results have brought some order into a picture that at one time seemed to be degenerating into cytotoxic anarchy.

In the mouse, Herberman and his colleagues have suggested that NK cells express low amounts of surface markers characteristic of T lymphocytes (Herberman *et al. J. Immun.* **121**, 304; 1978). Others, however, using even highly potent monoclonal anti-T cell antibodies, have not confirmed this. In any case, although the mouse Thy-1 antigen reportedly present on NK cells is characteristic of T and not B lymphocytes, it is also expressed in brain and other tissues, and its presence on NK cells would not necessarily link the NK effector with the T cell differen-

Killing comes naturally

from Peter Beverley & Dick Knight

tiation pathway. More persuasive in regard to the origin of the NK cells, however, are the observations that they carry a surface marker not present on T or B cells (although identified by a contaminant antibody in the anti-T cell serum anti-Lyt-1) and that the mutant *beige* mouse, which has deficient granulocyte function but normal T and B lymphocytes, has very low NK activity (Roder & Duwe *Nature* in the press).

In the human, recent work from Good's laboratory, as well as confirming the consensus that human NK cells possess receptors for the Fc portion of immunoglobulin, has suggested that both T and non-T cells are cytotoxic in NK assays (Gupta *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 5137; 1978). Moreover, suggestions are emerging that different target cells are differentially susceptible to the action of different effectors. In the mouse too, Stutman has recently described an NC (natural cytotoxic) cell with slightly different phenotypic properties and distinctly different killing preference to the classic NK cell (Stutman *et al. J. Immun.* **121**, 1819; 1978). If these results are confirmed, it becomes clear that both in mouse and man more than one effector cell may mediate 'natural' killing. In addition, since no convincing evidence for T-cell-mediated 'natural' killing has yet been presented for the mouse, a genuine species difference in effector compartments may exist.

Specificity can only be truly defined when the target antigens have been characterised. On the NK-susceptible mouse lymphoma target, YAC-1, three 'target structures' of molecular weights 130,000, 160,000 and 240,000, and distinct from known viral or histocompatibility antigens, have recently been identified (Roder *et al. Proc. natn. Acad. Sci. U.S.A.* in the press). On other targets, the available evidence from competition and monolayer absorption experiments suggests a broad selectivity of killing for groups of target cells as opposed to totally indiscriminate aggression. The emergence

of multiple NK effector populations, with apparent target preferences, especially in man, suggests further that not all NK cells will turn out to have the same range of specificities. However, the important question remains unresolved as to whether the observed selectivity reflects recognition of a limited number of ubiquitous antigens (and a fetal calf serum-derived antigen now seems unlikely), or the activity of many effector clones each recognising a single antigen.

What of specificity of immunisation? It is now clear that a variety of agents given *in vivo*—bacteria, viruses and tumour cells—can boost NK in mice. Interestingly, only those tumour cells themselves susceptible to NK lysis are able to augment NK activity in this way. A similarly wide range of agents boost NK *in vitro*, although the identity of the effectors of broad spectrum lysis induced after a period of culture with classical spontaneous NK, has not been proved. Recent work from Trinchieri and Santoli has shown, however, that *in vitro* inducers share the property of stimulating interferon release, and indeed, that interferon itself is a potent NK boosting agent (Trinchieri & Santoli *J. exp. Med.* **147**, 1314; 1978). No data has yet been presented on whether interferon is the only final common path of NK augmentation, or whether boosting *in vivo* and *in vitro* have the same mechanism.

Irrespective of whether interferon affects NK cells directly or works through an intermediate cell, it seems that the augmentation is the expression of a higher level of activity already programmed into the NK cells. This program depends not on the environment of the cell donor, but on its genetic constitution, since transfers of bone marrow cells (containing NK cells) between different mouse strains show that the transferred cells retain the level and spectrum of activity of the donor strain.

Genes controlling NK activity have been mapped close to genes controlling rejection of parent-into-F₁ bone marrow grafts and close to H-2D. Intriguingly, products of this latter locus are critical for target cell recognition in classical T-cell cytotoxicity. However, natural killing is not confined by self or even species recognition. If T cells are confirmed as an NK effector population, it is somewhat surprising that their

activity seems to have no MHC restriction.

Does this new information on the nature, specificity, and genetic control of the NK cell allow any tentative conclusions as to its function *in vivo*? Certainly data exist correlating tumour incidence and the ability to reject tumour grafts with the level of NK activity. Moreover, there seems to be a reciprocal relationship between T cell and NK activity, as athymic nude mice and thymectomised animals have higher levels of NK than intact syngeneic mice. The data from Kiessling's group, reported in this issue of *Nature* (page 174), showing that a subset of thymo-

cytes can be killed by NK cells, and correlating the presence or absence of this subset with the level of NK activity, also suggests that regulation of NK and T cells is closely connected. Should this subset of thymocytes turn out to be particularly susceptible to retransvirus-induced transformation, then the surveillance and immunoregulatory theories could be neatly fused. □

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A two-dimensional superfluid?

from P. V. C. McClintock

HAS two-dimensional superfluidity been observed? There has been a continuing controversy as to whether or not this interesting phenomenon, predicted theoretically for ideal two-dimensional systems, has been seen in real liquid helium-4 films. Dash has argued persuasively (*Phys. Rev. Lett.* **41**, 1178; 1978) that it has not; and that the experiments which had apparently demonstrated the effect were misinterpreted. Some new measurements now reported by Webster, Webster and Chester of the University of California at Los Angeles (*Phys. Rev. Lett.* **42**, 243; 1979) seem, however, to have tilted the balance of the evidence back in the affirmative direction once more, in favour of the original interpretation.

The superfluidity of the 'creeping film' which forms on surfaces dipping into liquid helium has, of course, been well known—and intensively studied—ever since it was first discovered by Rollin in 1936. It is this phenomenon which is responsible for the famous demonstration of superfluidity in which an open-topped cup of liquid helium is able to empty itself while remaining upright, using the film as a kind of syphon. The present interest centres, however, on very thin helium films: so thin, in fact, that no parameter can change within their thickness, and hence nothing can happen in the dimension at right angles to the surface on which the film is adsorbed. Such films probably constitute what are effectively two-dimensional (2-D) systems.

The theory of 2-D helium has made rapid progress in recent years under the

stimulus, particularly, of the beautiful experimental work carried out by Dash and his co-workers at the University of Washington, using exfoliated graphite as a substrate. A striking prediction made by Nelson and Kosterlitz (*Phys. Rev. Lett.* **39**, 1201; 1977) was that, if a 2-D system undergoes a superfluid transition, it will do so in a precipitous jump: the superfluid fraction ought to jump discontinuously from zero to a finite value, ρ_s^* . This is in sharp contrast to what happens in three dimensions. When ordinary bulk liquid helium undergoes the transition, the fraction of the liquid which behaves as superfluid increases continuously from zero. Nelson and Kosterlitz concluded, furthermore, that for a 2-D liquid helium-4 film the jump should be universal: that is, the ratio of ρ_s^* to the absolute temperature T_c at onset should be quite independent of the nature of the substrate, of the coverage at which the transition takes place, or indeed, of virtually anything else.

Apparent experimental verification of this remarkable prediction was reported shortly afterwards: by Rudnick, on the basis of third sound measurements (*Phys. Rev. Lett.* **40**, 1454; 1978); and by Bishop and Reppy, based on their measurements of the superfluid fraction on a vibrating substrate (*Phys. Rev. Lett.* **40**, 1727; 1978). The latter type of measurement seems at first sight to give a particularly direct and unambiguous measure of the superfluid fraction. The period of the oscillator is dependent on its mass, and thus on the effective mass of the adsorbed helium film sticking to it; but, if some proportion of the film is superfluid then, instead of following the oscillations, this fraction will just slip, and therefore will not affect the measured

period. Values of ρ_s^*/T_c deduced in this way were in excellent quantitative agreement with Nelson and Kosterlitz's prediction.

Dash argued, however, that the superfluid onset as measured by this technique does not represent genuine 2-D superfluidity. He suggested, first, that inhomogeneities in the Mylar substrate would cause the formation of 'puddles' of helium where the film was relatively thick, with interconnecting regions of thinner film. The observed superfluid onset therefore represented, not 2-D superfluidity in the film as a whole, but merely the occurrence of superfluidity in a sufficient proportion of the interconnecting regions. By this time the deep 'puddles' would, of course, correspond to 3-D superfluid. Second, he suggested that even if the substrate did provide an ideal atomically flat surface (which he doubted), the films themselves would be non-uniform. He envisaged that, as helium atoms were gradually added to the substrate, at a fixed temperature, they would first form a 2-D gas; but that, when a certain critical coverage was reached, interactions between the atoms would result in the formation of small patches of 2-D liquid surrounded by the 2-D vapour. As further atoms were added the connectivity of the liquid regions would eventually provide a continuous 2-D path across the whole experimental area and, if the temperature was low enough, superfluidity might then appear abruptly: the superfluid fraction at onset could clearly be non-zero, corresponding in fact to that of the liquid regions immediately before their being connected together.

In the experiment now reported from UCLA, the Websters and Chester have performed another vibrating substrate measurement, using an oscillating quartz crystal. The important difference from earlier work is that they have added an additional variable parameter to the system by mixing up to 30% of helium-3 with their helium-4: a proportion of helium-3 has a profound effect on the superfluid transition in helium-4, depressing it to lower temperatures and even eliminating it altogether if too much is added. Nonetheless, it turned out that the value of ρ_s^*/T_c was completely unaffected by the helium-3, remaining closely equal to the universal constant predicted by Nelson and Kosterlitz—although, of course, ρ_{tot} and T_c were individually changed to a considerable degree. So, for some as yet unexplained reason, Dash's arguments do not seem to be applicable in practice.

These latest results constitute compelling evidence in support both of the theory and of the belief that the helium-4 film at onset really does behave as a two-dimensional superfluid.

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Purine insertase: a new enzyme

from Gerard O'Donovan

It is now generally believed that a large number of environmental agents cause damage to DNA. Even in the absence of environmental stimuli DNA has to cope with yet another type of damage, namely the formation of DNA apurinic/apyrimidinic sites (AP sites). These lesions are produced spontaneously by the loss of purine or pyrimidine bases from the DNA and also by the action of a specific class of enzymes known collectively as DNA N-glycosylases (previously known as N-glycosidases). Three distinct enzyme activities have been discovered to date. These N-glycosylases remove uracil (Lindahl *Proc. natn. Acad. Sci. U.S.A.* **71**, 3649; 1974), 3-methyl adenine (Laval *Nature* **269**, 829; 1977; Riazuddin & Lindahl, *Biochemistry* **17**, 2110; 1978), or hypoxanthine (Karran & Lindahl, *J. biol. Chem.* **17**, 5877; 1978) from DNA without perturbing the sugar-phosphate backbone.

Until recently these enzymes were called DNA N-glycosidases because traditionally the base-sugar bonds in nucleic acids which they cleave, were termed glycosidic bonds (Lindahl, *Proc. natn. Acad. Sci. U.S.A.* **71**, 3649; 1974). However, the current rules for carbohydrate nomenclature (*Biochemistry* **10**, 3,983; 1971) state that a glycosidic bond is a linkage through oxygen obtained by replacement of the hydrogen atom of the hemiacetal hydroxyl group, while removal of the entire hydroxyl group results in a glycosyl bond (Riazuddin & Lindahl *op. cit.*).

Deoxyribonucleases that act on DNA containing apurinic sites have been characterised from various sources including bacteria (Gossard & Verly *Europ. J. Biochem.* **82**, 321; 1978), plants (Thibodeau & Verly *J. biol. Chem.* **252**, 3,304; 1977) and human cells (Kuhnlein *et al. Nucl. Acids Res.* **5**, 951; 1978). These enzymes are part of a duplex DNA repair pathway in which the apurinic endonuclease (AP endonuclease) makes an incision in the vicinity of the apurinic lesion, then the apurinic lesion is excised by an appropriate exonuclease and the resulting gap is filled in with a DNA polymerase before closure with DNA ligase. The apurinic lesion presumably arose spontaneously, by reaction with chemicals or as the product of a DNA N-glycosylase, one of the enzymes described above which removes ab-

normal bases from DNA by hydrolysing the glycosyl bond.

Since excision repair is a multi-enzyme process it seems reasonable that a simpler and more straightforward pathway might exist. With respect to modified DNA, N-glycosylolytic removal of abnormal bases from DNA to produce an AP site, with the concomitant reinsertion of the correct base, has been speculated on. In a recent issue of *Proc. natn. Acad. Sci. U.S.A.* (**76**, 141; 1979) Deutsch and Linn report the discovery of an enzyme activity from cultured human fibroblasts which seems to reinsert purines, but not pyrimidines, into DNA containing apurinic sites. The activity, which is partially purified (molecular weight 120,000) to be free from apurinic endonucleases, was first identified by its ability to bind specifically to partially depurinated DNA. Using radioactively labelled purines, Deutsch and Linn show that the free base is best utilised, and that the incorporation like the binding, is specific for DNA apurinic sites. Moreover, using depurinated synthetic polymers, they show that the insertion of purine bases seems to be template-specific, with guanine but not adenine being inserted into depurinated poly (dG-dC), and adenine but not guanine being inserted into poly (dA-dT).

The formation of a glycosyl bond would seem to require energy although the free energy of formation within a DNA molecule has not been measured directly. In the present study the reaction occurs in the absence of any obvious energy source. One suggestion offered by Deutsch and Linn for the free energy source in the case of a free purine substrate is the reforming of base pairs and stacking at the site of the reaction. With respect to an energy source, it is significant that another reaction in which the guanine base is utilised also occurs in the absence of energy. Here, the tRNA guanylation enzyme replaces the Q base of certain tRNAs with guanine (Okado *et al. Nucl. Acids Res.* **3**, 2,593, 1978; Farkas & Singh, *J. biol. Chem.* **248**, 7,781; 1977).

Clearly the most likely role for the AP endonucleases is in DNA repair. Such enzymes (purine base insertases) might be extremely active in repair during normal DNA replication. But because they are present in nature in such large amounts, almost universally, it is tempting to ascribe additional roles to the AP endonucleases. As pointed out by Deutsch and Linn it could be that the utility of base insertion may not be for DNA repair but rather for situations in which the generation of genetic diversity is desirable. In addition, the eukaryotic genome contains large numbers of modified bases which

could be replaced (or inserted) as a regulatory or developmental signal. Such hypotheses are simple to formulate but difficult to prove; it will be of interest to see if future investigations unmask more exotic DNA N-glycosylases and perhaps related insertases.

A surplus of carbon dioxide

from G. Skirrow

Two recent independent contributions (Brewer *Geophys. Res. Lett.* **5**, 997; 1978; Chen & Millero *Nature* **277**, 205; 1979), both based on analysis of Geosecs data, have outlined direct experimental evidence that part of the present, continuing, atmospheric CO₂ increase consequent on fossil fuel combustion is being transferred to the sea and that this process has probably been taking place for some time. Although it has always seemed reasonable to suppose that this transfer did occur, evidence has hitherto been mainly indirect and estimates of the extent of the exchange have relied on, for example, observations of the distribution of ¹⁴C produced by nuclear bomb explosions.

The air-sea transfer of CO₂ is merely one facet, albeit an important one, of the global carbon cycle. Briefly, CO₂ is gained by the atmosphere through volcanic exhalations, by evasion from the sea and by respiration and combustion. It is lost from the atmosphere by invasion into the sea, by photosynthesis into the terrestrial and marine biospheres and by weathering processes leading to dissolution of, particularly, carbonate rocks and the transfer to the sea of HCO₃⁻ rich solutions by continental run-off. Within the oceans biogenic precipitation of CaCO₃ from the supersaturated surface waters (together with precipitation of some organic debris) transfers some carbon to the sediments, although some of the CaCO₃ dissolves during its descent in the oceans. Over geological periods of time sea-floor spreading makes the sediments available for reworking. A more detailed examination shows that the carbon cycle is intimately linked with those for other elements, notably calcium, oxygen and nutrients.

Although life forms on Earth have changed over the past few hundred million years, the general and continuous persistence of life has been taken to imply that, over this time, the various elemental cycles have never been very violently perturbed from

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their steady states and that they have an essential stability deriving from in-built negative feedback or buffering mechanisms (Garrels *et al.* *Amer. Sci.* **64**, 306; 1976). Of course, many minor perturbations have occurred in the past although these have evidently not been sufficient to destroy life totally. However, even minor fluctuations may have consequences which, if not devastating, may be distinctly uncomfortable from a 20th century human standpoint. Thus, possible climatological changes may ensue. Quite apart from academic and intellectual reasons for wishing to understand more fully the natural processes of atmospheric and marine circulation and elemental cycle stabilisation, self-interest clearly justifies investing time, effort and money in monitoring the carbon system and in developing theoretical and modelling approaches intended to have interpretative or predictive value.

It is generally accepted that fossil fuel consumption is the main cause of the atmospheric CO_2 increase over the past century, although other human activities (deforestation and changing land usage) probably play a not insignificant part (Woodwell *Scient. Amer.* **238**, 34; 1978). However, there is no doubt about the reality of the partial pressure P_{CO_2} increase; precise and detailed measurements from many sites throughout the world over the past two decades (for example, Pales & Keeling *J. geophys. Res.* **70**, 6053; 1965) have shown that since 1958 atmospheric CO_2 has increased by about 6% and that the present atmospheric partial pressure of about 340×10^{-6} atm is some $50\text{--}60 \times 10^{-6}$ atm greater than that of a century ago. Precise knowledge of the CO_2 levels before 1850 (the effective start of the Industrial Revolution) is lacking but a value of about 290×10^{-6} atm is generally assumed. It is interesting to note that the observations made by Brewer and by Chen and Millero are at least not inconsistent with this picture, although at present the levels of precision imposed by the need to correct their basic data to allow for *in situ* CO_2 generation rules out definitive pronouncements.

The rate of increase of P_{CO_2} has increased with time in a manner matching the usage of fossil fuels, although a large fraction of the output has passed into other sinks—presumably the sea. The present annual rate of increase of P_{CO_2} is almost 1×10^{-6} atm yr^{-1} , and for the purposes of projection into the future (an uncertain business because of the likely dependence on economic factors and resource depletion) a rate of increase of something like 2% per year is often assumed (Broecker & Takahashi *The Fate of Fossil Fuel CO_2 in the Oceans*, 213,

Plenum, 1977). Sometime in the 21st century atmospheric P_{CO_2} may peak at several times the pre-industrial value (Woodwell).

Pertinent questions concern the way in which the sea responds to this increase of atmospheric partial pressure of CO_2 . That is, its capacity to moderate the increase by dissolution, the consequent changes in the chemistry of the sea, and the possibility that the attack of the now more aggressive waters on the calcite and argonite fractions of the sediments will further moderate the increase. The capacity of the sea to dissolve CO_2 is considerable although the time scale for total air-sea equilibrium is measured in centuries because of the relatively slow turnover and mixing times of the various oceanic water masses. However, according to Broecker and Takahashi who are grappling with the overall problem (including interaction with the sediments) if an injection to the atmosphere of 23×10^{16} moles of CO_2 is made, then on attainment of solution equilibrium about 70% of the injection would have passed into the sea. The effect of this uptake would be to moderate the carbonate ion concentration of the water to lead to dissolution of the CaCO_3 fractions of the sediments on certain parts of the ocean floor. Formidable problems make it difficult to quantify this general picture since these investigators have to contend not only with uncertainties concerning the future rate of industrial CO_2 generation and the rate of passage into the sea, but also with the complex kinetics of calcite dissolution (for example, Morse & Berner *Am. J. Sci.* **272**, 840; 1972). The difficulties are further compounded when it is recognised that (1) carbonate sediments are not uniformly distributed over the ocean floor, and the carbonate fraction varies considerably, falling virtually to zero for deep-sea sediments below the compensation depth; (2) the effectiveness of the carbonate fraction in neutralising the CO_2 increase depends on depth; at shallow depths the water is strongly supersaturated with respect to CaCO_3 and very high P_{CO_2} values are needed to remove this supersaturation (Skirrow & Whitfield *Limnol. Oceanog.* **20**, 103; 1975). Sediments lying between the present compensation and lysocline depths would be expected to be particularly susceptible to attack; and (3) as attack on the sediments proceeds, the remaining CaCO_3 will become more deeply buried or diluted by clay and inert materials thereby reducing the rate of attack.

Notwithstanding these discouraging considerations Broecker and Takahashi have made a start on this problem. They conclude that the potentially

available CaCO_3 in those sediments likely to be susceptible to attack is equivalent to the CO_2 derivable from the world's total fossil fuel resources and that the time constant for neutralisation of the CO_2 will be 1,500 years or less.

The studies referred to in the opening paragraph may seem a long way from those of Broecker and Takahashi, but they are different aspects of a large problem which may have important ramifications for the world as a whole. There are many grey areas in which information needs still to be collected. These include the response of the biosphere to changing atmospheric CO_2 levels, and possibly less important because of the already high levels of CO_2 in soil water, the effect this increased CO_2 will have on weathering processes. □

Making molecular catalogues

from Norman Anderson

HIGH-resolution two-dimensional gel electrophoresis, as originally described by O'Farrell (*J. biol. Chem.* **250**, 4007; 1975), has proved an extremely powerful tool for the analysis of complex protein mixtures, particularly cells. It can be made to resolve and detect quantitatively virtually all the proteins expected in *E. coli* (~ 1,500) and perhaps 2,000–3,000 of the possible 4,000–7,000 thought to occur in any given mammalian cell type. So far, however, the technique has been principally used as an adjunct to studies of particular enzymes or regulational events, and has not provided the kind of global information that might be expected to emerge from a detailed analysis of what different cells do (or at least synthesise). The reason for this is the difficulty of comparing large numbers of patterns, and of remembering enough differences to draw conclusions with regard to very large numbers of protein spots. A somewhat analogous state of affairs existed in early protein crystallography when diffraction spots could be produced on X-ray film, but the structure of the proteins producing them could not be deduced. The introduction of computers, automated densitometers, and mathematical analysis ultimately allowed such structures to be determined. This process seems to be repeating itself, but at a much higher level of complexity, in the field of high resolution two-dimensional protein mapping.

What is desired is a way of reducing

2-D gel data to quantitative lists of protein components (that is, precise spot positions and integrated densities), and ways of matching and comparing lists to deduce the rules or factors governing the synthesis of each gene product. An early and relatively easy project, for example, would be to determine the number of protein gene products common to all cell types in man, or conversely, whether proteins exist which are absolutely specific to each differentiated cell type. In order to address such questions, computer-based data reduction systems are being constructed by several groups in the US and Europe. An informal meeting at the Argonne National Laboratory reviewed progress and discussed systems design with likely major users.

J. Garrels (Cold Spring Harbor Laboratory) and N. Xuong and M. Miller (University of California, San Diego) described working systems for spot detection and integration based, respectively, on slope and contour analysis. The latter seems much faster but requires interactive help in separating compact groups of spots. In each of these systems, some progress has been made towards matching pairs of gels for comparison. Two examples of second generation dedicated mini-computer systems were described by L. Anderson and J. Taylor (Argonne) and J. Garrels (CSHL). The first segment of the two-part Argonne system (called TYCHO) involves image scanning, spot detection, and quantitation by two-dimensional Gaussian fitting, matching of patterns to compensate for small differences between gels by interactive colour television graphics, and the production of spot lists containing as many human protein gene products (PGPs) as possible. The program for searching the spot lists for correlations with the type or state of the cells producing them is called KEPLER. The Cold Spring Harbor program (called QUEST) will use virtually identical hardware but is designed primarily to compare patterns as raw image data rather than spot lists to facilitate accurate ratio quantitation in double-label autoradiographic experiments. Both systems aim to accumulate a comprehensive annotated map (or list) of proteins in a particular organism (man at Argonne and mice and rats at Cold Spring Harbor) for general research use.

An important constraint in such systems (particularly when applied to large-scale genetic work) will be processing time per gel. This is currently estimated at 20 to 60 min in the second

generation systems, depending on the number of spots. A faster image processing technology based on the Sternberg-von Neumann cytocomputer (described by P. Petraitis, Environmental Research Institute, Michigan) seemed capable of reducing processing time by at least 50%, while solid array based densitometer designs (P. Spragg, University of Birmingham, UK) offered similar improvements.

The potential use of these systems for detecting possible changes in the human background mutation rate was discussed by J. V. Neel (University of Michigan). On the basis of the current Argonne throughput of approximately 10,000 2-D gels per year, and assuming that several hundred protein gene products could be examined in each person, mutation rates could be estimated on samples as small as 30,000 individuals in a reasonable time.

Preliminary results presented by E. McConkey (University of Colorado, Boulder) indicated a wide discrepancy in average heterozygosity between major cellular proteins (as observed on 2-D gels) and the proteins of plasma and red cells (measured by classical methods). This sparked a lively debate on the effects of natural selection on different groups of proteins and brought out the view that many geneticists are still convinced that charge-change variants are better detected on starch blocks than on 2-D gels. This question was settled by the development by N. L. Anderson and B. H. Hickman (Argonne) of charge shift standards prepared by progressive carbamylation of one protein. These standards stretch all the way across the gel as a series of discrete spots, one more than the number of amino groups on the original protein, and unequivocally demonstrate the detectability of unit charge shifts.

P. O'Farrell (University of California, San Francisco) described experiments to determine whether repressed mammalian genes are indeed totally silent. Using a combination of immunoaffinity and two-dimensional separations, he was able to place an upper limit of one part in 2×10^8 on the expression of a particular protein in cells carrying the corresponding gene in a repressed state. Interestingly enough, any gene products present at this level *in vivo* are less abundant than mismatched versions of actin and other major proteins, due to the limited fidelity of translation. There is, therefore, an irreducible 'background fog' associated with high resolution protein mapping at currently achievable sensitivities as a result of thermodynamic noise in protein synthesis.

It was generally concluded that

within the year systems will exist capable of cataloguing, comparing, and performing perturbation analyses on a good fraction of the possible products of a mammalian genome. By extending the set of observable proteins from the classically assayable (presently perhaps 2% of the total) to the most abundant 50%, we will probably be afforded a new view of many biological phenomena and, perhaps, a less welcome look at how poorly controlled present experiments are at the level of gene expression. □

Saccharin: from carcinogen to promoter

from E. Boyland

THE first indications that the widely used artificial sweetener saccharin was carcinogenic were the data of 1951 in which there was "an increased incidence of the ordinarily uncommon condition of abdominal lymphosarcoma" in rats fed saccharin¹. When the technique of implantation of pellets containing suspected direct carcinogens into the bladder of mice was introduced², it was thought that, as the bladder tissue would be less able than the liver to metabolise foreign compounds, the test would distinguish compounds that required metabolic activation from those which were directly carcinogens. The implantation of cholesterol pellets containing saccharin into the urinary bladders of a few mice induced a statistically significant incidence of tumours³. At the time we suggested that "The induction of bladder tumours with saccharin suggests that the presence of the solid pellet in the bladder may have a promoting action and that the method of bladder implantation detects incomplete carcinogens". These results with saccharin were confirmed 13 years later by experiments carried out on a larger scale⁴. Previously it had been found that saccharin had some initiating action on mouse skin⁵, and so the bladder implantation experiments in the light of knowledge at that time were interpreted as showing that saccharin was a direct carcinogen and active without metabolic activation. Subsequent investigations have been unable to detect any metabolic changes of saccharin in any of several species^{6,7}.

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Following the bladder implantation experiments and results, at least eleven studies in which saccharin was fed to rats are available⁸; in eight of these urinary bladder tumours seem to have been induced, but in never more than 44% of rats and generally in a much lower proportion even when the diets contained 5% saccharin. In one study⁹ lymphomas and leukaemias occurred.

Saccharin may therefore be considered as a weak carcinogen for the rat bladder, by the following three criteria. (1) The dose needed to induce carcinogenesis was large—in some cases of the order of 1 kg per kg body weight. (2) The induction time was long; generally the bladder tumours appeared after at least 18 months continuous feeding. (3) Not all animals developed tumours. Potent carcinogens on the other hand induce cancer in all treated animals.

Later experiments may explain the mode of action of saccharin. When small 'initiating' doses of methyl nitrosourea (NMU) were instilled into the urinary bladder of Wistar rats which were then given either water or water containing saccharin, to drink, no bladder tumours were seen in rats given NMU without saccharin¹⁰. Only 2% of rats given saccharin alone developed bladder tumours in 95 weeks, but 57% of rats given NMU and saccharin developed tumours. Some of the rats with tumours had bladder stones but these were not considered a factor in the 'syncarcinogenic' effect of saccharin. The authors concluded that saccharin is cocarcinogenic and "that a promoting action of saccharin with cyclamate and/or other bladder carcinogens is an obvious possibility".

In another experiment the feeding of diets containing 2% FANF (N-(4-5 nitro-2-furyl 2-thiazoyl) formamide) to rats for 6 weeks induced bladder cancer in 4 out of 20 rats, but if this was followed by feeding 2% tryptophan or 5% saccharin 20 out of 39 and 35 out of 35 rats developed bladder cancer¹¹. In these experiments both tryptophan and saccharin seem to have acted as promoting agents. The result with tryptophan was similar to those in which rats fed 2-acetamidofluorene with either tryptophan or indole developed bladder tumours, whereas with 2-acetamidofluorene alone tumours at other sites were seen^{12,13}.

Further evidence that saccharin acts as a promoting agent was provided by the experiments in which cultures of C3H/10T1/2 cells, treated with non-transforming initiating doses of methylcholanthrene followed by saccharin, had a statistically significant number of transformations¹⁴. These authors estimated that saccharin probably functioned as a promoting agent, but was

1,000-fold less active than the potent promoter 12-O-tetradecanoyl-phorbol-13-acetate.

If saccharin is a promoter rather than a direct carcinogen, how can the earlier results be explained. The first bladder implantation experiments in animals showed that although the rat skin was resistant to the carcinogenic action of tar the epithelium of the rat bladder was sensitive¹⁵. Later experiments¹⁶ however, showed that implantation of paraffin wax pellets alone into the rat bladder produced a high incidence of cancer. Mice treated in the same way developed only a few bladder tumours and so could be used for carcinogen testing by this technique. The wax pellets therefore seem to be acting as promoters and rat urine or bladder epithelium may contain more material acting as initiators of carcinogenesis than does that of mice.

All the experiments in which bladder cancer has been induced by feeding saccharin have been in rats. The occurrence of these bladder tumours could therefore be due to the promoting action of saccharin together with endogenous initiators of carcinogenesis.

Several experiments therefore indicate that saccharin could be a promoting agent rather than a complete carcinogen. Saccharin is not mutagenic in the *Salmonella* reverse mutation assay of Ames or the cell transformation test of Styles and there is no indication that it is metabolised by animals or man or reacts with DNA. It has been suggested that as the carcinogenic action in rats and the dominant lethal effects in mice do not appear to be genetic; they are 'epigenetic'¹⁷. This term is a broad one and would include

promoting action, cocarcinogenesis, inhibition of DNA repair and suppression of immune reactions. The experimental data on saccharin indicate that it is a promoting agent and this is more precise than saying its effects are not produced by a genetic mechanism. The local effect of saccharin could be due to its being concentrated in urine and the bladder mucosa.

However this is not evidence that saccharin is 'harmless'. Carcinogenesis is a complex multistage process in which initiation, promotion or some other stage may be limiting steps. Thus although promoting agents are not complete carcinogens, in some conditions they facilitate cancer development which would not otherwise occur. In such circumstances, a promoter may therefore be as dangerous as a complete carcinogen. Saccharin is, however, unlikely to provide a serious hazard because the dose required to produce the promoting effect is so large—of the order of 1 kg per kg body weight. The very low though finite risks may be worth the benefits of its use. □

Fossil pseudo-reptiles

from Barry Cox

IN Kipling's *Just So Stories*, the jaguar was dreadfully puzzled by the armadillo, for he wasn't sure whether it was a tortoise or a hedgehog. In the same way, the tiny Carboniferous tetrapods were for long a puzzle to palaeontologists, who couldn't tell whether they were amphibians or reptiles.

There was obviously no way in which their life-history could be established. It also became clear that the simple-minded assumption that any wholly terrestrial tetrapod was a reptile, while any partially aquatic tetrapod was an amphibian, wouldn't work either: there was too much ecological overlap in these early tetrapods. So palaeontologists had to look for osteological criteria on which to distinguish these animals, though their small size made this difficult—nearly all are under 30 cm long, and some are less than 10 cm long. The most reliable characteristic was found to be the structure of the vertebral centra: these seemed to have ossified in one block in the little tetrapods that were eventually called the Lepspondyls, while there were usually two pairs of ossifications in the larger forms known as Labyrinthodonts.

It eventually became clear that the lepospondyls included three distinct groups. The aquatic neotrideans had an elongate, deepened swimming tail,

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and the limbless aistopods looked very like the living apodans. The bulk of the lepospondyls, which lacked these specialisations, were left in the order Microsauria. Although increasing understanding of early reptile evolution made it possible to identify several small reptilian genera and remove them from the Microsauria, the order itself remained poorly defined and lacking any comprehensive, well-illustrated descriptive and analytic review. Such a review has now been provided by Robert L. Carroll and Pamela Gaskill (*Mem. Amer. phil. Soc.*, 126, 1; 1978).

Carroll and Gaskill recognise 25 genera in the order Microsauria, including five new genera. Various characters of the skull, cranio-cervical joint and scales are diagnostic of the group, but the trunk vertebrae of some are similar to those of reptiles in having small intercentra. For many years, the best-known genus was *Microbrachis*; its clear lateral-line canals and external gills, elongate body, small limbs and three-fingered manus were therefore used as the model for the restoration of other, less complete microsauro genera. However, it is now clear that *Microbrachis* is a member of a sub-order of aquatic microsauria that were much less varied than the remainder of the group. The members of the larger sub-order, which Carroll and Gaskill call the Tuditanomorpha, show considerable variation in the size and proportions of the body, and in the dentition.

The microsauria occupied a number of different habitats. As well as the microbrachids, some other genera seem to have lived in ponds or streams (although not in deeper water), but the short body and massive limbs of genera such as *Tuditanus* suggest that they were wholly terrestrial. Microsauria were covered by bony scales and heavy, double-headed ribs which may have been used in costal ventilation of the lungs. There is therefore no reason to suppose that they were as ecologically restricted as the scale-less, moist-skinned amphibians of today. Microsauria were probably carnivorous; a number of genera have rather massive teeth, and probably preyed on molluscs or arthropods that had hard shells or carapaces.

Microsauria are known only from the Pennsylvanian (Late Carboniferous) and Early Permian. They first appear alongside the first reptiles in the Early Pennsylvanian, but the microsauria were at that time considerably more diverse than the reptiles, suggesting

that they had originated earlier and had had more time to diversify. But the reptiles quickly became the dominant group, and the microsauria that survived into the Permian were mainly of the heavy-toothed, elongate type that looked less like reptiles and presumably competed less with them. The geographical distribution of microsauria, to the adjoining continents of North America and Europe, is also notable. Carroll and Gaskill think that there is no particular significance in this fact, pointing out that Carboniferous deposits laid down on land or in shallow water are rare or unknown in Gondwanaland and Asia.

The general similarity in size and mode of life of microsauria and modern amphibians makes it difficult to be sure

whether morphological similarities between the two groups indicate relationships or merely convergence. Although the Early Permian labyrinthodont *Dolesempetron* has been suggested as a possible ancestor of modern amphibians, Carroll and Gaskill point out that its only specific similarity is its possession of pedicellate teeth. There is still so great a gap in time between the earliest members of the modern amphibian orders and any of the Palaeozoic forms that there was ample time for their evolutionary transformation from any small Palaeozoic amphibian that had not already undergone divergent specialisations such as the loss of limbs. Only new Permian or Triassic discoveries are likely to solve this problem. □

Irreversible segregation in irradiated alloys

from R. W. Cahn

THE elementary theory of atomic diffusion shows that a solid solution, even if thermodynamically stable, cannot be in a state of complete equilibrium unless the concentration of solute attains a state of flat uniformity. The elementary theory of diffusion is always wrong, because no real solid is structurally uniform. Real pieces of alloy have free surfaces and contain grain boundaries, dislocations and other defects. It is well established that solute segregates to such singularities, and the degree of segregation in equilibrium depends in particular on the solubility of the solute: relatively insoluble species segregate more intensely. The temperature is the other major variable, and a lower temperature spells more segregation. This reversible process is known as equilibrium segregation; it has been intensively studied because of its practical implications, some (by no means all) deleterious. Increased intergranular corrosion and reduced ductility in creep are examples of consequential disadvantages, sintering of 'doped' alumina powder to give a translucent, pore-free solid is an instance of beneficial use. This scientifically most intriguing as well as practically important phenomenon has recently received a worthy review by Hondros and Seah (*Int. Metals Rev.* 22, 262; 1977). Equilibrium segregation is not, however, the sole form of segregation in solid solutions.

The alternative form is non-equilibrium or irreversible segregation, and it is associated with flows of point defects, lattice vacancies in particular. Such flows result when a solid is quenched from a high temperature so that it becomes super-saturated with vacancies, or else when it is being continuously

irradiated, with the consequential creation of a steady supply of vacancies and interstitial atoms. The flow of excess point defects is absorbed at a 'sink' (such as a free surface, grain boundary or dislocation) and the defects then disappear. If, in some way, the moving defects carry solute atoms along with them, then the resultant segregation is plainly irreversible, because it is brought about by a non-equilibrium, unidirectional process—the flux of point defects.

The study of this kind of segregation has been going on for about 10 yr and reviews are beginning to appear. An early account was by Anthony, who charmingly entitled it 'Atom Currents Generated by Vacancy Winds' (in *Diffusion in Solids* (ed. Nowick & Burton) Academic Press, 1975); a more comprehensive treatment, including the more recent irradiation studies which only began when Anthony was writing his review, was presented by Harries and Marwick of Harwell at a conference on 'Residuals, Additives and Materials Properties' in May 1978 (to be published by the Metals Society, London). With the help of some very recent papers, the true complexity as well as the practical implications, for nuclear reactors, of irreversible segregation is now becoming clear, and will be outlined here.

The experimental facts are that, under irradiation, solutes (both major and minor) segregate irreversibly to or from a free surface. The experiments are usually done with ion irradiation instead of neutrons, to hasten the

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process. This was discovered in 1974; a recent study is by Marwick and Piller (*Rad. Effects* **33**, 245; 1977) who found in a Ni-Mn-Si alloy that manganese concentrated in the vacancy-rich layer below the surface (the vacancy 'source') whereas silicon moved out of this layer towards the sinks; the segregation was very pronounced indeed. The same investigators (*J. Nucl. Mater.* **71**, 309; 1978) confirmed the segregation of nickel in a Fe-Ni-Cr alloy to the surface sink.

It has also been found that similar segregation takes place near the surface of the tiny voids that form in some metals and alloys, over a restricted temperature range, during irradiation. (These voids result from the fact that point defect sinks are apt to absorb interstitials slightly faster than vacancies, and so those vacancies which survive both recombination with interstitials and absorption at sinks, finish up in these minute, empty holes). This segregation process was first found in 1977 in aluminium (see account in *Nature* **262**, 671; 1977) and has now been confirmed by an exceedingly painstaking electron microprobe study at Harwell (Marwick *et al. Scripta Met.* **12**, 1014; 1978). Since the void diameter is mostly in the 100–200 nm range and the irradiation-induced segregation is confined to a layer adjacent to each void of about 100 nm thickness, the utmost resolution of a modern scanning electron microscope was required to demonstrate the segregation, and a number of possible errors had to be checked for. Nevertheless, the findings are quite unambiguous: in the Fe-Ni-Cr-Mo alloy used, the Ni/Fe and Ni/Cr ratios (these being the three major elements) are enhanced near the voids. This matches the earlier finding, that nickel is enriched near surface sinks.

These findings are by no means straightforward to interpret, because the theorist is faced by an embarrassment of alternative transport processes. The most obvious is by the formation of vacancy-solute and interstitial-solute associations; vacancies and self-interstitials in the host lattice become locked to solute atoms with variable binding energies, and the bound solutes are then dragged along with the point defect flux. The most comprehensive attempt to interpret in detail the recent findings in these terms is by Lam, Okamoto and Johnson (*J. Nucl. Mater.* **73**, 408; 1978), who improved a model they had been developing for several years. To make sense of the motion of various solutes either with the point defect flux (that is, to sinks) or against that flux (that is, to regions of peak radiation damage), they were forced to postulate very complex patterns of association; for instance, interstitial-solute associations were assumed to

differ so much in binding strength that some are readily mobile while others cannot move. There are too many adjustable parameters for comfort.

A novel alternative theory has been advanced by Marwick (*J. Phys. F: Metal Physics* **8**, 1849; 1978). Marwick concentrates attention on what he terms the 'inverse Kirkendall effect'. In the normal diffusional Kirkendall effect in a binary solid solution, found in the absence of irradiation, the vacancy flow is governed by the relative partial diffusion rates of the two constituents as they mingle with each other (that is, desegregate). The excess vacancies may form voids near the diffusion interface. Under irradiation, Marwick points out, the situation is stood on its head: a vacancy flow is imposed, and any disparity of partial diffusion rates leads to segregation of solute. The segregation process then creates a countervailing vacancy flux tending to cancel out that generated by irradiation—le Chatelier's Principle in operation. Marwick analyses the process in detail and proves that it is not at all necessary to postulate point defect-solute binding: segregation can result from the inverse Kirkendall effect alone. The faster diffusing species moves up the vacancy gradient (away from sinks) at a rate proportional to the difference between the two diffusivities; the slower diffusing species drifts to the sinks. This model makes sense of the observed enrichment of nickel, a slow diffuser, at surfaces and voids. More generally, Marwick postulates that the inverse Kirkendall effect largely governs the behaviour of concentrated alloy constituents while point defect-solute associations are likely to be the more important feature for dilute solutes.

Much interest has been expressed in the implications of irreversible segregation for void-swelling (Marwick *op. cit.*; Harries, *Nucl. Energy* **17**, 301; 1978). Structural materials and cladding in fuel assemblies for fast reactors are subject to slight but potentially dangerous swelling as voids form in those parts of the assemblies which are set at the relevant temperatures. Intensive research over the past few years has led to the use of cold-worked austenitic nickel-rich alloys (containing Fe+Ni+Cr+Si+Ti) which have excellent swelling resistance at all temperatures. It may now be possible to improve these alloys even further in the light of the new information on segregation, because it is clear (especially if the inverse Kirkendall effect is dominant) that the enrichment of slow-diffusing species, especially nickel, near incipient voids must impede further vacancy access, both because of the preponderance of a species in which vacancies move slowly and also because of the inverse vacancy flux (the balmy

vacancy wind) induced by the inverse Kirkendall effect. It seems likely that vacancy access to voids may be choked off almost completely, and some further accurate segregation tests with various solutes may point the way to even more effective anti-void measures. Minor solute elements may have a disproportionate effect if, as is quite possible, their presence has a large effect on the partial diffusivities of the major constituents and thus on the magnitude of the inverse Kirkendall effect. Future improvements may open up the way to fast reactor alloys containing less nickel than the present preferred alloy; nickel soaks up an undue number of neutrons and other alloys might be preferable from the viewpoint of neutron economy.

Another possibility is to prevent void formation altogether by using a non-crystalline alloy in which, presumably, no conventional vacancy flux can arise. Reichtin, Van der Sande and Baldo (*Scripta Met.* **12**, 639; 1978) have just reported that glassy Nb₄₀Ni₆₀ shows no void formation on heavy ion irradiation, and recommend that glasses like this be explored for use in nuclear reactors. □



A hundred years ago

IN the December part of the *Transunti* of the Royal Academy dei Lincei of Rome, Prof. Cossa gives an interesting account of his researches on the occurrence of the three metals cerium, didymium, and lanthanum. It appears that although these metals occur always in but minute quantities, yet their occurrence is far more frequent than is generally supposed, Prof. Cossa having been able to trace them even in bones and in the ashes of plants, not to speak of a number of minerals, such as certain apatites, Carrara marble, scheelite, &c. In Carrara marble Prof. Cossa found about two centigrammes of the mixed oxalates of cerium, lanthanum, and didymium in every kilogramme of marble; there were also traces of yttrium.

FURTHER experiments were made last Thursday in lighting the British Museum reading-room with the electric light. The result showed that by proper arrangement and at a comparatively moderate cost, there is good reason to believe that the end desired can be obtained. The Paris Société Générale d'Electricité have made the experiments at their own cost.

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review article

γ -Ray line astronomy

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γ -Ray astronomy is a valuable source of information on solar activity, supernovae and nucleosynthesis. Cosmic γ -ray lines were first observed from solar flares and more recently from the galactic centre and a transient event. The latter may give an important insight into nuclear reactions taking place near neutron stars and black holes and a measure of the gravitational redshifts of such objects.

γ -RAY lines are the most direct probe of cosmic nuclear processes. While it has been recognised^{1,2} for two decades that nuclear γ -ray spectroscopy could provide basic information on such problems as solar activity, supernova dynamics and nucleosynthesis, only recently have positive observations of celestial γ -ray lines been made.

The first cosmic γ -ray lines were observed³ from solar flares. These lines are produced in nuclear processes on time scales of several minutes by the interaction of flare-accelerated particles with the solar atmosphere. Solar γ -ray observations have provided⁴ important information on particle acceleration mechanisms, and on the flare process itself.

The strongest observed⁵ steady celestial γ -ray line is at 0.511 MeV resulting from positron annihilation. Many mechanisms can contribute to galactic positron production, and two of these, energetic particle reactions and nucleosynthesis, also produce other detectable lines. As we shall show, the bulk of the positrons responsible for this annihilation radiation may come from processes of explosive nucleosynthesis in supernovae. Such positrons result from relatively long-lived radioisotopes whose decay is accompanied by several strong de-excitation γ -ray lines. These lines have not yet been detected, but the predicted fluxes are within the range of instrumentation planned for forthcoming γ -ray observations. The measured 0.511-MeV line intensity already places constraints on the present rate of nucleosynthesis in the Galaxy.

Cosmic γ -ray lines from a transient event have recently been observed⁶. These lines seem to result from energetic particle reactions similar to those that take place in solar flares, but not in similar environments. The identification of the lines requires a redshift of a magnitude that suggests the strong gravitational fields of compact collapsed objects. Such γ -ray line observations may probe nuclear reactions taking place in the vicinity of neutron stars and black holes, and measure the gravitational redshifts of such objects⁷.

We first discuss the flare observations and their implications. The study of γ -ray lines from flares is important not only for gaining unique insights into the flare process, but also for the development of the theory of astrophysical γ -ray line production.

Solar γ rays

Solar γ -ray lines were first observed³ from the 4 and 7 August 1972 flares with a NaI(Tl) detector on the 7th Orbiting Solar Observatory (OSO 7). For the 4 August flare, the observed lines, at 2.22, 0.51, 4.44, and 6.13 MeV, and the continuum above 1 MeV, had fluxes of 0.28, 0.06, 0.03, 0.03, and 1 photons cm⁻² s⁻¹ respectively. The duration of the event was about 10³ s. Lines at 2.22 and 0.51 MeV were also detected during the decay phase of the 7 August flare, but only upper limits could be set on the 4.44 and 6.13 MeV lines and the continuum from this flare. Observations of the 2.2-MeV line have been reported^{8,9} from the flares of 22 November 1977 and 11 July 1978.

All these lines result from nuclear interactions of flare-accelerated particles most probably in the chromosphere or lower corona. The strongest line, at 2.223 MeV, is due to neutron capture on hydrogen, $^1\text{H}(n, \gamma)^2\text{H}$. The neutrons with initial energies of 1–100 MeV, are thermalised and captured in the photosphere. The width of the 2.223-MeV line, determined by the photospheric temperature, is very narrow (~ 100 eV). Neutron capture on ^1H , however, must compete⁴ with capture on ^3He , even though ^3He is only a minor constituent of the photosphere, because the cross-section for the reaction $^3\text{He}(n, p)^3\text{H}$ is about four orders of magnitude larger than that for the reaction $^1\text{H}(n, \gamma)^2\text{H}$. Observations of the 2.223-MeV line intensity set a limit on the photospheric $^3\text{He}/^4\text{He}$ ratio of several times 10⁻⁴; this limit is comparable to the measured $^3\text{He}/^4\text{He}$ ratio in the chromosphere and solar wind (see ref. 10).

Radiative neutron capture on ^{56}Fe , which produces two strong lines at 7.632 and 7.646 MeV, is also of considerable astrophysical interest⁷. These lines should be dominant in an iron-rich medium such as the surface of a neutron star.

The second strongest line from solar flares is at 0.511 MeV from positron annihilation. The principal sources of positrons in flares are π^+ mesons, short-lived radioactive positron emitters such as ^{11}C , ^{13}N , ^{14}O and ^{15}O , and the first nuclear level of ^{16}O at 6.052 MeV which decays by electron-positron pair emission. The initial energies of the positrons range from several hundred keV to tens of MeV, but only a small fraction annihilate at these high energies. The bulk of the positrons slow down to energies comparable with those of the ambient electrons, where annihilation takes place either directly or from bound states of

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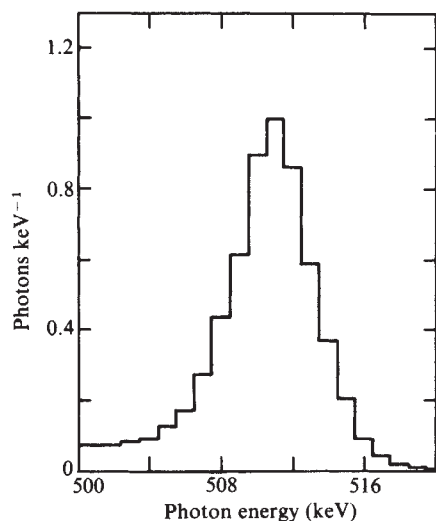


Fig. 1 Calculated¹² spectrum of positron annihilation radiation in cold atomic hydrogen.

positronium. Positronium is formed by radiative combination with free electrons and by charge exchange with neutral hydrogen; 25% of the positronium annihilates from the singlet state and 75% from the triplet state.

Direct annihilation produces two 0.511-MeV photons in the positron-electron rest frame. Singlet positronium also annihilates into two such photons, but triplet positronium decays into three photons which form a continuum at energies below 0.511 MeV. This continuum, however, is significant only if the ambient density is less than about 10^{15} cm^{-3} so that triplet positronium is not broken up by collisions before it can annihilate.

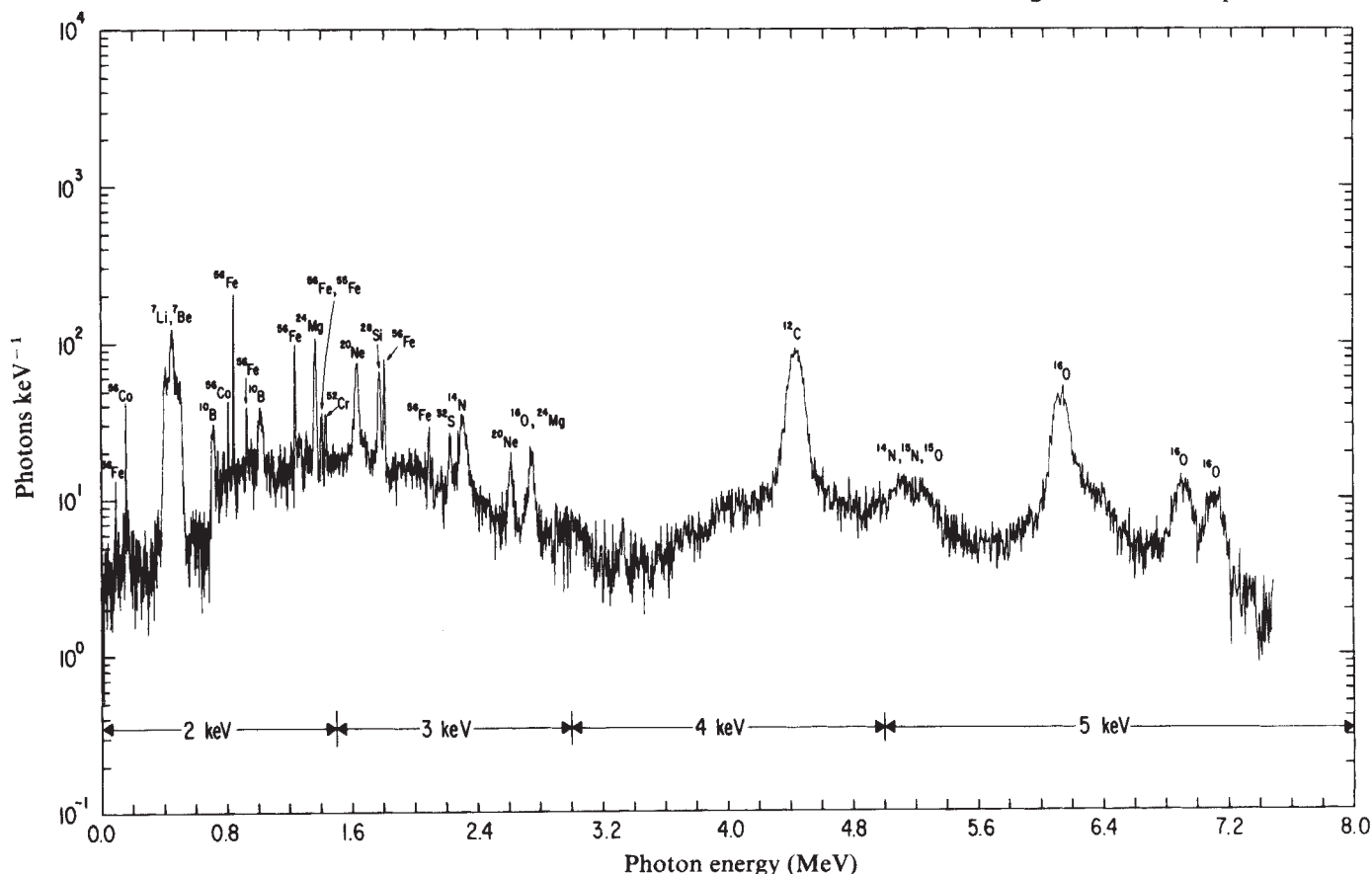
If the ambient medium is almost neutral ($n_e/n_H \leq 0.05$), about 95% of the positrons form positronium in flight. If on the other hand, the medium has a higher degree of ionisation, energy loss to the plasma thermalises¹¹ the positrons before they can annihilate directly or form positronium. In this case, direct annihilation dominates if the temperature is greater than about 10^6 K , while at lower temperatures the positrons form positronium by charge exchange and radiative combination.

If the density of the ambient medium is sufficiently low ($\leq 10^{15} \text{ cm}^{-3}$), the spectrum of the annihilation radiation in a neutral medium depends on the kinetic energy of the positronium formed in flight and on the positron-neutral atom interaction leading to direct positron annihilation. This spectrum, obtained¹² from a Monte Carlo simulation, is shown in Fig. 1. The full width at half maximum (FWHM) of the 0.511-MeV line is $\sim 5 \text{ keV}$, and the asymmetry due to triplet positronium annihilation can be clearly seen.

In an ionised medium, the width of the 0.511-MeV line depends mostly on the temperature; the FWHM is about 3 keV at 10^5 K and it varies approximately as the square root of the temperature. Note that in an ionised medium the 0.511-MeV line can be substantially narrower than in a neutral medium. For example, in a partially ionised warm gas at 10^4 K (such as in a young supernova remnant) the width of the line is $\sim 1 \text{ keV}$, while in a cold neutral medium (such as an interstellar cloud) the width is $\sim 5 \text{ keV}$.

The expected width of the 0.511-MeV line from solar flares could range¹¹ from a few keV to several tens of keV depending on whether the annihilation takes place predominantly in the cool photosphere or the hot flare plasma. Because of its low energy resolution, the NaI(Tl) detector on OSO 7 has set only an upper limit ($\sim 40 \text{ keV}$), but future high resolution observations, by measuring the line width, may determine the predominant positron annihilation site.

In addition to neutron and positron production, energetic particle reactions lead to many other lines resulting from de-excitation of nuclear levels. Figure 2 shows the spectrum from



such de-excitation, calculated¹³ by a Monte Carlo simulation of an energetic particle population with spectrum proportional to E^{-2} interacting with a cold ambient medium. Here E is energy per nucleon, and both the energetic particles (at the same E) and the ambient medium are assumed to have solar composition. The narrow lines result from the de-excitation of ambient heavy nuclei excited by energetic protons and α particles, while the underlying broad component is due to de-excitation of energetic heavy nuclei interacting with ambient H and He. The strongest narrow lines, at 4.44 and 6.13 MeV, due to ^{12}C and ^{16}O de-excitation, respectively, were observed³ from the solar flare of 4 August 1972. Because the ratio of the intensities of the 4.44- and 2.223-MeV lines depends strongly on the spectrum of the energetic particles, the observed ratio provides information on this spectrum in the flare region⁴. The broad component, as well as other narrow lines which were not resolved in the 4 August 1972 observations, could account^{14,15} for all of the observed radiation above 4 MeV. Several other narrow lines could also be detected from large flares with instrumentation of higher sensitivity than that flown on OSO 7, for example, the 0.847-MeV line (Fig. 2) from ^{56}Fe and the 15.11-MeV line¹⁶ from ^{12}C .

An important result¹⁰ of solar γ -ray studies concerns the acceleration of protons, nuclei and electrons to energies greater than about 1 MeV. It seems that the mechanism for this acceleration differs from the bulk energisation of solar electrons to tens of keV where they are manifest in powerful hard X-ray emission. Acceleration to higher energies (≥ 1 MeV), on the other hand, is probably due to a stochastic Fermi-type process which imparts $\sim 1\%$ of the flare energy to a small fraction of the ambient particles. This acceleration may be similar to galactic cosmic-ray acceleration, as several of the properties of the energetic solar particles, such as chemical composition and proton-to-electron ratio, resemble those of the cosmic rays.

Steady galactic γ -ray lines

The best observed^{5,17} nontransient galactic γ -ray line is at 0.511 MeV from the galactic centre. The observed⁵ line at 510.7 ± 0.5 keV has an intensity of 1.2×10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$ and FWHM < 3.2 keV. This observation was made in 1977 with a balloon-borne high purity Ge detector of opening angle 15° centred on the galactic centre. The line energy clearly identifies it as positron annihilation radiation; there is also some evidence for the three-photon continuum from triplet positronium annihilation.

The observed spectrum⁵ is shown in Fig. 3; the solid curve is the sum of the extrapolated low energy continuum (dashed curve) and a three-photon continuum assuming $\sim 90\%$ annihilation by positronium. This fraction is consistent with positron annihilation in a low density ($< 10^{15} \text{ cm}^{-3}$) and low temperature ($< 10^6 \text{ K}$) medium¹², and implies about 0.6 photons in the 0.511-MeV line per positron.

The 3.2-keV upper limit on the width of the 0.511-MeV line suggests¹² that the bulk of the positrons annihilate in an ionised medium ($n_e/n_H \geq 0.05$), because in cold interstellar clouds, the line width (~ 5 keV, Fig. 1) would exceed the observed upper limit. Possible sites of annihilation could be supernova remnants, H II regions at the galactic centre and the warm ionised component of the interstellar medium. Observations with higher spatial resolution could help determine the nature of source.

Turning now to the origin of the positrons, we first consider π^+ mesons produced in high energy (> 100 MeV) cosmic ray interactions in the interstellar medium. The π^+ mesons, produced in proton-proton and proton-helium interactions, decay through μ^+ mesons into positrons. An upper limit on this positron source can be set from high-energy (> 100 MeV) γ -ray observations¹⁸, as at least part of these γ rays should result from the decay of π^0 mesons which are also produced in the above interactions. From the measured π^+ and π^0 production cross

sections, we find that the observed¹⁸ flux ($\sim 3 \times 10^{-5}$ photons $\text{cm}^{-2} \text{s}^{-1}$) of > 100 MeV γ rays from a 15° cone centred on the galactic centre implies that not more than about 2% of the observed⁵ 0.511-MeV line can be from the annihilation of positrons from π^+ decay.

There is probably also a distinct low-energy (< 100 MeV) cosmic-ray component; the interstellar density of this component, however, is not known because solar modulation excludes the bulk of the low-energy particles from the inner Solar System. γ -Ray line production from these cosmic rays has been recently evaluated¹³ under the assumption that their local interstellar density is 1 eV cm^{-3} , and that there are substantial gradients along the line of sight towards the galactic centre in both the cosmic-ray energy density and heavy element abundances. The resultant γ -ray line emission, including the 0.511-MeV line shown in Fig. 4, is close to its maximal allowed value. If the cosmic-ray intensity were increased, X-ray line emission at ~ 6.8 keV due to charge exchange of fast Fe ions with interstellar matter would exceed the observational upper limit on this line, and if the heavy element abundances along the line of sight were increased, the absorption of X-rays would be larger than observed. The 0.511-MeV line intensity from low-energy cosmic rays, therefore, should not be larger than $\sim 7 \times 10^{-5}$ photons $\text{cm}^{-2} \text{s}^{-1} \text{ rad}^{-1}$, or also about 2% of the observed line intensity in a 15° cone around the galactic centre.

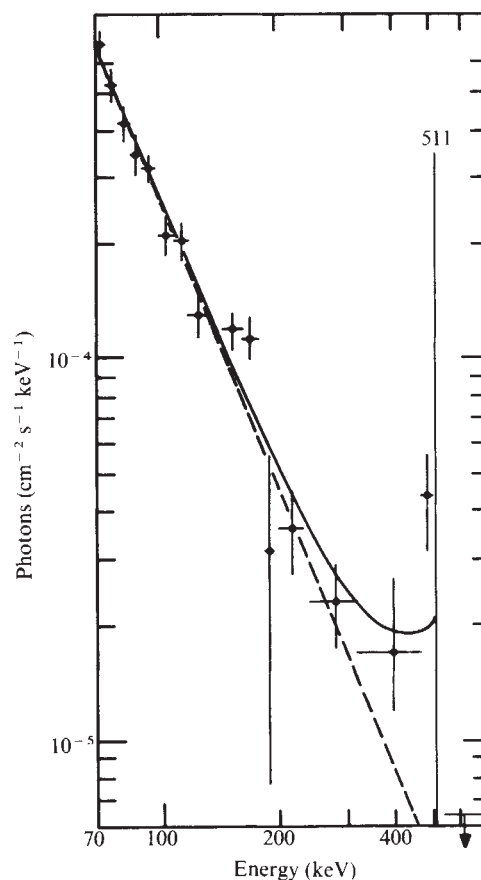


Fig. 3 Observed⁵ 0.511-MeV line and low-energy γ -ray spectrum from the galactic centre.

Low-energy cosmic-ray interactions in the interstellar medium, nevertheless, produce a rich γ -ray spectrum which includes many nuclear de-excitation lines, as can be seen in Fig. 4. Of special interest are the very narrow lines, such as that at 6.129 MeV from ^{16}O , resulting from de-excitation of nuclei in interstellar grains¹⁹. The line broadening, which in gases is caused by the recoil velocities of the excited nuclei, is greatly reduced in solids where these nuclei or their radioactive

progenitors can come to rest before de-excitation. The detection of γ -ray lines from low-energy cosmic-ray interactions in the interstellar medium with forthcoming instrumentation would not only measure the unknown interstellar density of these cosmic rays, but also provide unique information on the distribution, motion, composition and size of interstellar dust grains.

Returning to the question of the origin of the positrons, energetic particle reactions at low energies (<100 MeV) could produce positrons in discrete sources (such as in the vicinity of a massive black hole at the galactic centre⁷) without violating the constraints set by the X-ray observations¹³. If this is the case, the 0.511-MeV line should be accompanied by strong broad line emission from nuclear de-excitation, in particular the 4.44-MeV line from ^{12}C . This line may have been observed¹⁷ in 1974 with a balloon-borne NaI (TI) detector, but this measurement was not confirmed by recent observations (J. L. Matteson, personal communication) with the first High Energy Astronomical Observatory (HEAO 1). The discrepancy could be due to time variations which will be further explored with this and forthcoming satellite-borne γ -ray spectrometers.

If, however, the nuclear interaction rate is essentially constant over the positron annihilation time, then the HEAO 1 upper limit on the 4.44-MeV line intensity from the galactic centre would require that not more than about 30% of the observed 0.511-MeV line result from such energetic particle reactions. This conclusion is only weakly dependent on the assumed composition of the ambient medium and the energetic particles, since the major contributors to positron production, C, N, and O, also produce 4.44-MeV line emission by either direct excitation or spallation. The energetic particle spectrum also does not greatly affect the positron-to-4.4-MeV photon ratio¹³. Thus, some other galactic positron sources may also be required to account for the observed 0.511-MeV line.

One such source^{2,20,21} could be relatively long-lived positron-emitting radioisotopes produced by nucleosynthesis during the explosive ejection of matter in supernova explosions. The decay of these isotopes is accompanied by de-excitation γ -ray lines. The principal isotopic decay chains are shown in Table 1, together with the relevant mean lives, de-excitation line energies, and number of positrons and photons per disintegration. The isotopic yields, normalised to ^{56}Ni , are based on Solar System abundances²⁴, and on the assumption^{22,23,25} that all of the Solar System ^{56}Fe , ^{57}Fe and ^{44}Ca , and 1%, 0.5%, and 0.1% of the ^{60}Ni , ^{22}Ne and ^{26}Mg , respectively, are produced explosively through the decay chains shown in Table 1.

Even though nucleosynthesis takes place in dense regions which are opaque to γ rays, the expanding envelope can become transparent if there is sufficient delay between the synthesis of the isotopes and the emission of the photons. For the de-excitation lines, the delay is due only to the lifetime of the parent isotope. But for the 0.511-MeV line, the delay can be longer because of the finite duration of positron slowing down and annihilation. Thus, even relatively short-lived isotopes such as ^{56}Co may be important sources of positrons for annihilation radiation.

Although the lifetime of ^{56}Co may not be long enough for the supernova to become transparent to its de-excitation lines, a significant fraction of the positrons from ^{56}Co may escape from the supernova²⁰ and annihilate in regions which are transparent to the γ rays. Furthermore, even if transparency is achieved by sufficiently rapid expansion of the supernova envelope and low ejected mass, the de-excitation lines from ^{56}Co decay could only be seen for a short time (a few years) after the explosion. Therefore, these lines would be observable mostly from extragalactic supernovae from which the expected fluxes are relatively small. The slowing down and annihilation times of the positrons, however, can be longer than the interval between supernova explosions²¹, and this could lead to an essentially steady galactic emission at 0.511 MeV. The time dependences and detectability of the various other de-excitation lines listed in Table 1 have been discussed in detail elsewhere²³.

Table 1 Isotopic decay chains from nucleosynthesis in supernovae

Decay chain	Mean life (yr)	$Q/Q(^{56}\text{Ni})$	Positrons or photons per disintegration	
$^{56}\text{Ni} \rightarrow ^{56}\text{Co} \rightarrow ^{56}\text{Fe}$	0.31	1	e^+	0.2
			0.847 MeV	1
			1.238 MeV	0.7
$^{57}\text{Co} \rightarrow ^{57}\text{Fe}$	1.1	2×10^{-2}	0.122 MeV	0.88
			0.014 MeV	0.88
$^{22}\text{Na} \rightarrow ^{22}\text{Ne}$	3.8	5×10^{-3}	e^+	0.9
$^{44}\text{Ti} \rightarrow ^{44}\text{Sc} \rightarrow ^{44}\text{Ca}$	68	2×10^{-3}	1.275 MeV	1
			e^+	0.94
			1.156 MeV	1
			0.078 MeV	1
			0.068 MeV	1
$^{60}\text{Fe} \rightarrow ^{60}\text{Co} \rightarrow ^{60}\text{Ni}$	4.3×10^5	1.5×10^{-4}	1.332 MeV	1
			1.173 MeV	1
			0.059 MeV	1
$^{26}\text{Al} \rightarrow ^{26}\text{Mg}$	1.1×10^6	1.5×10^{-4}	e^+	0.85
			1.809 MeV	1

It has been estimated²⁰ that $\sim 10\%$ of the positrons from ^{56}Co decay could escape from the supernova. This escape fraction would make ^{56}Co the dominant positron source for processes of nucleosynthesis, as can be seen from Table 1. The production of $\sim 2 \times 10^{43}$ positrons s^{-1} in the Galaxy, as indicated by the 0.511-MeV line observations⁵, implies a present rate of nucleosynthesis of $\sim 10^{45}$ iron nuclei s^{-1} . This is $\sim 10\%$ of the average rate of nucleosynthesis over the age of the Galaxy, and is consistent with current models²⁶ of galactic chemical evolution. The fraction of escaping positrons from supernovae is very uncertain, however, and requires further theoretical study. If the escape fraction for ^{56}Co were much lower than 0.1, ^{22}Na decay could become the dominant positron source, as can be seen from Table 1. But the ^{22}Na yield from supernovae²⁵ is also very uncertain. ^{22}Na is also produced in novae, with a yield of about 10^{48} nuclei per nova explosion²⁷. To produce $\sim 2 \times 10^{43}$ positrons s^{-1} in the Galaxy, however, ~ 600 novae per year would be required, and this rate is higher by an order than current estimates of the galactic nova rate.

As there are other potential positron sources, a test of the explosive nucleosynthetic origin would be the detection of the associated de-excitation γ -ray lines. A good prospect is the 1.809-MeV line from ^{26}Al decay²⁸. Because of the long lifetime of this isotope, the 1.809-MeV line should be observed as steady galactic emission with an intensity of $\sim 5 \times 10^{-5}$ photons $\text{cm}^{-2} \text{s}^{-1} \text{rad}^{-1}$ from the direction of the galactic centre; the long half-life of the ^{26}Al also implies a very narrow width for this line (~ 3 keV).

Another possible positron source could be pair production in the magnetosphere of pulsars²⁹. But observations of the Crab Nebula have so far not detected line emission at 0.511 MeV from this object. Although a line at 0.400 MeV, attributed to gravitationally redshifted 0.511-MeV emission was detected³⁰ from the general direction of the Crab, this line was not seen⁶ in an earlier flight which viewed the same region. This suggests that the 0.400-MeV line from the direction of the Crab could be due to a transient event.

Transient γ -ray lines

In addition to steady γ -ray line emission exemplified by the 0.511-MeV line from the galactic centre, γ -ray lines have also been observed from transient events. γ -Ray bursts of duration up to tens of seconds are a well recognised cosmic phenomenon, but their energy spectra have not yet been measured with sufficient resolution to discern whether they contain significant line emission. There seems, however, to be another class of

γ -ray transients in which essentially all the observed radiation is in the form of lines. Such a γ -ray line transient was discovered⁶ with a balloon borne Ge(Li) detector on 10 June 1974. This event, lasting ~ 20 min was characterised by strong emission in four relatively narrow energy bands or lines with no detectable continuum. The four observed lines at energies of 0.40–0.42, 1.74–1.86, 2.18–2.26 and 5.94–5.96 MeV had average intensities of 7×10^{-3} , 3.2×10^{-2} , 1.5×10^{-2} and 1.5×10^{-2} photons $\text{cm}^{-2} \text{s}^{-1}$, respectively, during the event. These lines showed statistically significant increases in emission compared both to the intensity in the adjacent continuum and to that in the same energy bands before and after this event. A simultaneous increase was observed in the CsI(Na) shield detectors with an intensity consistent with that observed just in the lines. This transient source seems to lie within $\sim 20^\circ$ of RA 7h and dec 20° . One possible candidate for the source is the object $\gamma 195+5$ which has been observed³¹ in high-energy γ rays but not yet at other wavelengths. The Crab Nebula was also near the edge of the field of view.

There are no simple nuclear schemes that can account for all four observed lines, as there are no obvious candidate nuclei for direct emission at ~ 0.41 and 5.95 MeV. A source seems to be required⁷ which is effectively bimodal, producing both redshifted and non-redshifted lines. The observations could then be understood in terms of neutron capture and positron annihilation, which are also the strongest line producing mechanisms in solar flares. Specifically, the ~ 0.41 -MeV line could be redshifted positron annihilation radiation and the 1.79- and 5.95-MeV lines could be redshifted neutron capture on ^1H and ^{56}Fe , respectively. The required redshift Z of between 0.24 and 0.28, could arise from the gravitational field of a neutron star of mass 1.4 – $1.8 M_\odot$. Episodic accretion onto such a neutron star from a close binary companion could lead to the formation of a high temperature ($>10^{10}$ K for ions) accretion disk in which

nuclear interactions could take place producing neutrons and positrons. Positron annihilation and neutron capture on iron and hydrogen at and near the surface of the neutron star would produce the observed redshifted line emission, while the same processes in the accretion disk and in the atmosphere of the companion star would produce essentially unshifted lines, of which only the 2.223-MeV line from neutron capture on hydrogen was observed. The unshifted 0.511-MeV positron annihilation line was not seen because of the large atmospheric and detector background at this energy. The double line from neutron capture on iron should be significant only in the redshifted emission from the iron-rich surface of the neutron star but not in the unshifted emission.

As mentioned above, the 0.400-MeV line observed³⁰ in May 1976 from the general direction of the Crab Nebula may also have been a γ -ray line transient as it was not detected⁶ in an earlier flight. The June 1974 and May 1976 events may in fact have the same source, as both observations included much of the same field of view; the latter event, however, was weaker by a factor of 3.

Extragalactic γ -ray lines

γ -ray lines at 4.4 and 1.6 MeV have been reported³² from the radiogalaxy Centaurus A. The 0.511-MeV line, however, which is so prominent in the spectra of solar flares and the galactic centre, was not observed from Cen A. If the γ -ray lines from Cen A are produced⁷ in quasithermonuclear reactions in the vicinity of a massive black hole³³, then the absence of the 0.511-MeV line could be due to the large Doppler broadening in the hot accretion gas which would make the line unobservable above the continuum. γ Rays in the MeV region have also been reported³⁴ from the Seyfert galaxy NGC4151, but the energy resolution was not sufficient to resolve possible line emission.

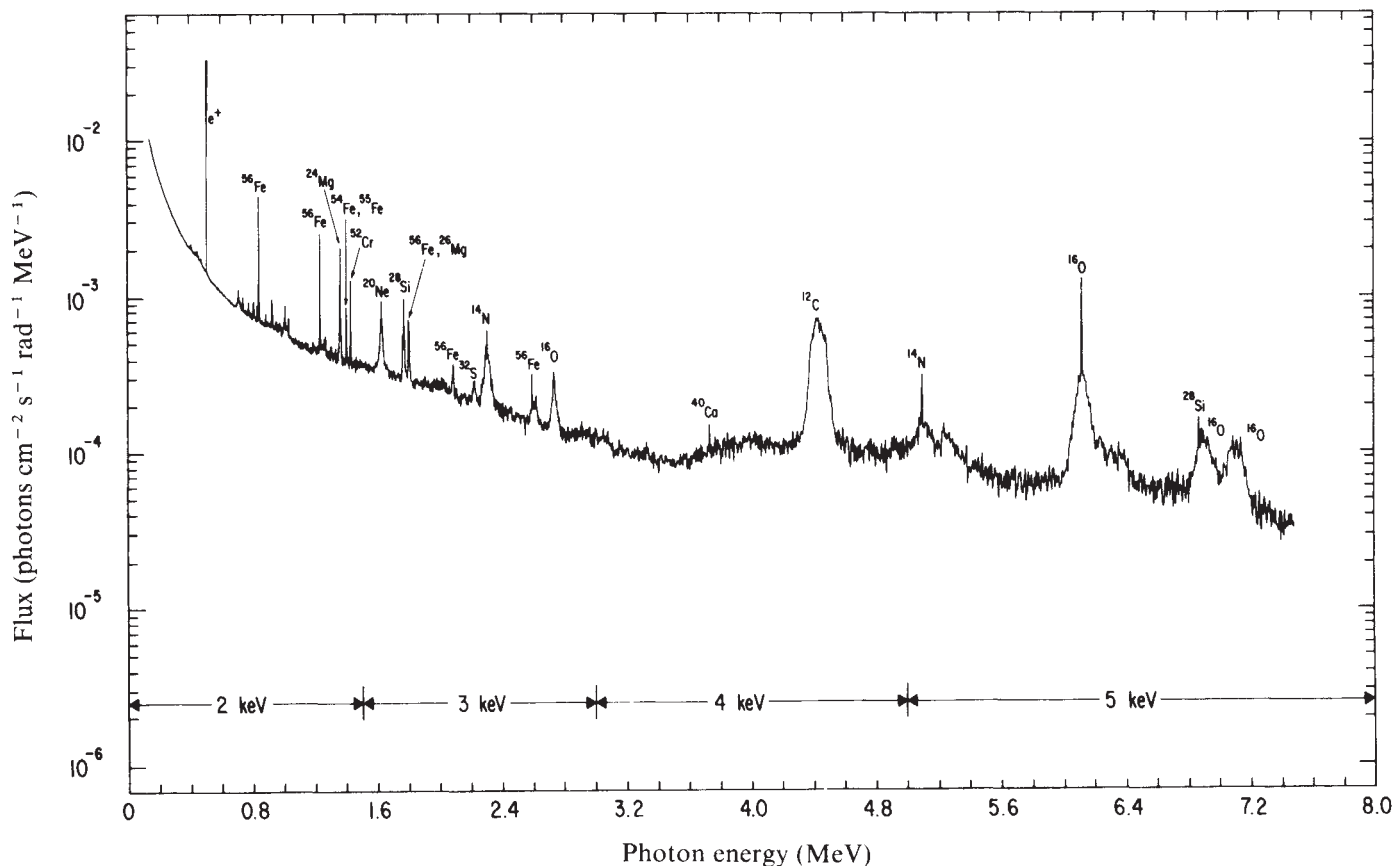


Fig. 4. Expected¹³ γ -ray spectrum from low-energy cosmic-ray interactions in the galaxy in the general direction of the galactic centre.

Outlook

Information on many astrophysical process and sites is encoded in the γ -ray line observations reviewed here. Following these exciting discoveries, γ -ray line astronomy is now moving into an era of more systematic study, made possible by the launch of HEAO 1 and the anticipated launches of HEAO C and the Solar Maximum Mission (SMM) in the autumn of 1979. The γ -ray spectrometer on HEAO 1 is already giving results. Within a year, solar flares will be explored with the γ -ray spectrometer³⁵ on SMM, and galactic and extragalactic γ -ray lines will be studied by a high resolution spectrometer³⁶ on HEAO C. At the same time, high altitude balloon-borne spectrometers should continue to provide important results on discrete sources.

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Looking further into the future, perhaps the most exciting prospect for γ -ray line astronomy will come with the launch of the Gamma Ray Observatory in 1984. This observatory is not yet an approved mission. Two of its five experiments, a Ge spectrometer and a NaI spectrometer, will observe γ -ray lines with much higher sensitivities than the instruments flown to date or planned for the immediate future. Together, these satellite and balloon-borne experiments should establish γ -ray line astronomy as a vigorous observational science.

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articles

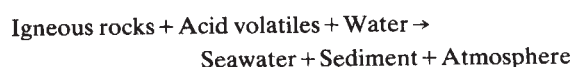
Water-rock partition coefficients and the composition of seawater and river water

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Using a simple chemical reactor model a strong correlation is established between the mean oceanic residence time of an element and the partitioning of that element between seawater and crustal rock. The ocean-rock partition coefficient ($K_{Y(SW)}$) is shown to be related to the electrostatic contribution to the element-oxygen bond energy (Q_{YO}) and a second correlation is established between these parameters. In this way the concentrations of all the elements so far identified in seawater fall in a coherent pattern. The global mean composition of river water, which represents an intermediate step in the formation of seawater from rock weathering, is shown to conform to a similar pattern.

THE concentrations of nearly 70 elements have now been determined in seawater and, although the chemistry of seawater has been frequently reviewed^{1–3} no general rules have emerged that enable the concentrations of all the elements to be incorporated in a simple yet coherent pattern. According to one hypothesis^{4–6}, seawater has been produced by the reaction,



As there have been no major changes in the mean composition of either the igneous rocks or the sediments over the past few billion years^{4,7} the composition of seawater has probably remained fairly constant over this period—in order of magnitude terms at least. Aided by the vast flux of material through the oceans^{5,7} and by the uplift and reweathering of sedimentary rock^{7,8} (sometimes following metamorphism) the ocean-rock

system has run so close to a steady state that, for more than 60 elements the rate of input to the oceans (by river transport) is almost exactly balanced by the rate of removal (by sedimentation)⁹. As the oldest marine sediments are no more than a few hundred million years old it is possible that the same material has passed through the oceans several dozen times during the Earth's history. This suggests that it should be possible to use the partitioning of the elements between the oceans and the crustal rocks to provide further insight into the nature of seawater itself.

Before we can draw meaningful conclusions from such an exercise we must consider the reactivity of the elements in seawater because, as Forchhammer¹⁰ pointed out in 1865, "the quantity of the different elements in seawater is not proportional to the quantity of the different elements which river water pours into the sea, but inversely proportional to the facility with which elements in seawater are made insoluble by general chemical or organochemical reactions in the sea". The modern approach to Forchhammer's concept of chemical reactivity in the oceans is to define a mean oceanic residence time ($\bar{t}_Y$) (ref. 11). Elements will have a short residence time if they are readily incorporated in hydrogenous mineral phases and hence rapidly removed from the oceans. Over the 2,000 Myr period that we postulate for our steady-state ocean, the water has been stirred at close to 1,000 revs Myr⁻¹. If we approximate the behaviour of this well-stirred system by a continuously stirred tank reactor (or ocean bucket) which includes the world's estuaries we can use the notation of chemical reactor theory¹² to define $\bar{t}_Y$ so that

$$\bar{t}_Y = M_Y / \bar{m}_Y \quad (1)$$

where M_Y is the total mass of Y dissolved in the oceans^{11,13} and $\bar{m}_Y$ is the mean flux of Y through the oceans in unit time. If $\bar{t}_Y$ is less than the time (t_R) required for one stirring revolution of the reactor then $\bar{t}_Y$ can not strictly be interpreted as a residence time.

The weathering cycle and oceanic residence times

To relate mean oceanic residence times to the weathering cycle we introduce a crude model. The ocean bucket will be considered as one component in a sequence of interlinked reservoirs^{14,15} where the rate of transport of material from one reservoir to the next is directly proportional to the total amount of Y in the source reservoir. The subscripts A, B and C will represent the crustal rock, river water and oceanic reservoirs respectively. If we assume that the oceans are at a steady state with respect to the flux of dissolved components and that the composition of reservoir A remains constant, it can be shown that, for first order transfer kinetics over a period that greatly exceeds the residence time of elements in reservoir B,

$$k_C \bar{Y}_C N_C = k_B \bar{Y}_B N_B = k_A \bar{Y}_A N_A$$

so that

$$\bar{t}_Y = \bar{Y}_C N_C / k_B \bar{Y}_B N_B = \bar{Y}_C N_C / k_C \bar{Y}_C N_C = \bar{Y}_C N_C / k_A \bar{Y}_A N_A \quad (2)$$

N_X is the anhydrous mass (kg) of the reservoir X and $\bar{Y}_X$ is the global mean concentration ($\mu\text{mol per kg solids}$) of the element Y

in the reservoir X so that $M_Y = N_C \bar{Y}_C$. k_X is the first order rate constant for transfer of material from reservoir X. Taking logarithms in equation (2) we find that

$$\log \bar{t}_Y = \log K_{Y(\text{sw})} - \log (k_A N_A / N_C) \quad (3)$$

where

$$K_{Y(\text{sw})} = \bar{Y}_C / \bar{Y}_A$$

Equation (3) suggests that there should be a simple relationship between the partitioning of the elements between the oceans and the crustal rocks (resulting from the long-term recycling of the system) and the mean oceanic residence times (which reflect the reactivity of the individual elements in the oceans). Using tabulated values of $\bar{Y}_A$ (ref. 16), $\bar{Y}_B$ (corrected for recycled sea spray)^{17,18} and $\bar{Y}_C$ (ref. 13) we can calculate $\log \bar{t}_Y$ and $\log K_{Y(\text{sw})}$ and produce a plot (Fig. 1) showing a significant correlation for more than 40 elements that can be summarised by the equation

$$\log \bar{t}_Y = a_0 \log K_{Y(\text{sw})} + b_0 \quad (4)$$

a_0 does not have the expected value of unity (Table 1) but this is not surprising considering the crudity of the model. The $\bar{Y}$ -values used to characterise the reservoirs are at best only order of magnitude estimates for most of the elements and no account has been taken of the individual chemical behaviour of the various elements. Despite these limitations only three elements fall clearly outside the correlation. Nitrogen enters the oceans as nitrate but, rather than accumulating in the oceanic reservoir it is converted to gaseous nitrogen under anoxic conditions and is then lost to the atmosphere. An ocean at thermodynamic equilibrium would contain 0.1 M nitrate ion^{8,21}. To account for the behaviour of this element and also for that of carbon, a fifth reservoir (the atmosphere) should be introduced into the model. Because of this anomalous behaviour, nitrogen was omitted from the statistical analysis of Fig. 1. For silver and antimony quite large discrepancies arise between the residence times calculated from the input of dissolved solids by the world's rivers¹⁷ and those calculated from the formation of hydrogenous sediments^{22,23}. If the sedimentation values, rather than the river input values, are plotted for these elements the points fall within the correlation limits (Fig. 1, arrowed data points). Only five elements (Fe, Al, La, Zr and Pb) have residence times that are less than t_R and these elements form a tightly grouped set in Fig. 1. From tabulated values of $\bar{Y}_A$ (ref. 16) and $\bar{Y}_C$ (ref. 13) equation (4) can be used to calculate mean oceanic residence times for elements not included in Fig. 1 that should be accurate to within an order of magnitude.

The importance of the residence time concept for estimating the response of the oceans to changes in the rate of input of material has recently been re-emphasised²⁴. Because the time span of the injection must be greater than t_R for a valid application of the residence time concept, the use of $\bar{t}_Y$ -values for predicting the effects of anthropogenic inputs over the next few centuries must be treated with caution. However, these values should be useful for discussing the stability of ocean chemistry in the geological past.

Table 1 Linear regression fitting parameters for the correlations discussed in the text ($y = ax + b$)

Fig. no.	Elements considered	n^*	y	x	a	b	$Sy, x^\dagger$	$r^\ddagger$	$r_{0.1}^\S$
1	All except N	44	$\log \bar{t}_Y$	$\log K_{Y(\text{sw})}$	0.59 ± 0.05	6.25 ± 0.16	0.77	0.88	0.52
2	Na, K, Rb, Cs	4	$\log K_{Y(\text{sw})}$	Q_{YO}	-3.27 ± 0.77	23.12 ± 5.62	0.59	0.95	0.999
2	Li, Mg, Ca, Sr, Ba	5	$\log K_{Y(\text{sw})}$	Q_{YO}	-1.68 ± 0.55	9.65 ± 3.42	0.67	0.87	0.99
2	Main body of elements	60	$\log K_{Y(\text{sw})}$	Q_{YO}	-1.21 ± 0.10	0.68 ± 0.36	1.23	0.85	0.42
3	Li, Na, K, Rb, Cs, Mg, Ca, Sr, Ba	9	$\log K_{Y(\text{rw})}$	Q_{YO}	-0.59 ± 0.21	4.27 ± 1.38	0.44	0.73	0.95
3	Main body of elements	36	$\log K_{Y(\text{rw})}$	Q_{YO}	-0.77 ± 0.12	2.36 ± 0.34	0.95	0.75	0.55

* Number of data points.

† Standard error of estimate of y on x .

‡ Correlation coefficient.

§ Value of r that must be exceeded for the correlation to be significant at the 0.1% level²⁰.

|| This correlation is significant at the 5% level.

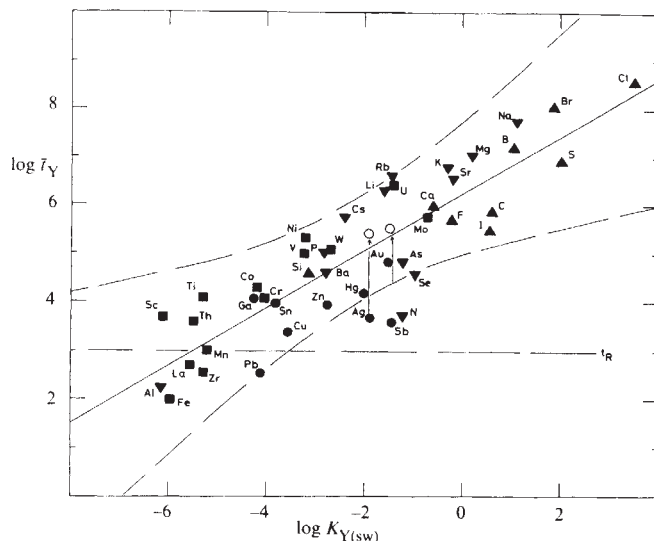


Fig. 1 The relationship between the mean oceanic residence time ($\bar{t}_Y$) and the seawater-crustal rock partition function ($K_{Y(sw)}$) of the elements. ∇ , pre-transition metals; $\blacksquare$, transition metals; $\bullet$, B-metals; $\blacktriangle$, non-metals. Open symbols indicate $\bar{t}_Y$ -values estimated from sedimentation rates. The solid line indicates the linear regression fit (Table 1) and the dashed lines the Working-Hotelling confidence band at the 0.1% significance level¹⁹. Nitrogen was omitted from the statistical analysis for reasons given in the text. The horizontal broken line indicates the time required for one stirring revolution of the ocean (t_R). (The source data used in the preparation of Figs 1 to 4 could not be included in this paper because of space limitations but they are available in tabulated form on request from the authors.)

Relationship between composition of river water and seawater

Rather than attempting to develop a more complex model to account for the observed values of a_0 and b_0 (equation (4), Table 1) we will treat equation (4) as an empirical relationship and will focus instead on the factors controlling $K_{Y(sw)}$. Substituting $\bar{t}_Y$ from equation (2) into equation (4) we can show that

$$\log(\bar{Y}_C/\bar{Y}_B) = a_0 \log(\bar{Y}_C/\bar{Y}_A) + b'_0 \quad (5)$$

where

$$b'_0 = b_0 + \log(k_B N_B/N_C)$$

This equation is an empirical relationship independent of the multiple reservoir model. Subtracting $\log K_{Y(sw)}$ from both sides of equation (5) results in

$$\log K_{Y(rw)} = (1 - a_0) \log K_{Y(sw)} + b'_0 \quad (6)$$

where $K_{Y(rw)} = \bar{Y}_B/\bar{Y}_A$. Equation (6) suggests that river water represents an intermediate stage in the formation of seawater from the weathering of rocks, and that the partitioning of elements between the solid and aqueous phases is controlled by the same factors in each case. The controlling factor cannot generally be precipitation of simple salts (such as hydroxides, and carbonates) as solubility product calculations show that the majority of the elements in seawater have lower concentrations than the limit set by their most insoluble compounds^{22,25}. Schindler²⁶ has suggested that adsorption-desorption reactions at the surfaces of minerals which are essentially oxide-dominated matrices (mainly silicates, aluminosilicates, oxides, hydroxides and carbonates) control the concentrations of many trace metals in seawater. He has produced some convincing calculations for mean oceanic residence times on the assumption that the rate of removal of trace elements from the oceans is controlled by adsorption reactions at the surface of amorphous

silica. In this and other oxide matrices the elements attach themselves to the mineral surfaces through the deprotonated surface hydroxyl groups²⁶. This is essentially an ionic interaction, the strength of which can be related to the electronegativity of the element in question²⁷.

Electronegativity and the partitioning of elements

Pauling²⁷ suggested that the nature of the chemical bond formed between any two atoms could be related to the ability of the individual atoms to attract the electrons available for bond formation. To this end he formulated an electronegativity scale that provided a crude but effective measure of the electron attracting capabilities of the elements. The measured energy of the Y-O bond is found to exceed the arithmetic mean of the Y-Y and O-O bond energies by a quantity Q_{YO} which can be related to the electronegativities of the elements (x_Y and x_O respectively) by

$$Q_{YO} = (x_Y - x_O)^2 \text{ eV} \quad (7)$$

Q_{YO} is therefore a measure of the electrostatic contribution to the Y-O bond energy and we will use Q_{YO} as a measure of the attraction that an oxide-based mineral lattice will exert on the element Y. Using tabulated electronegativity values^{16,28} we can plot $\log K_{Y(sw)}$ (Fig. 2) and $\log K_{Y(rw)}$ (Fig. 3) against Q_{YO} . In accordance with equation (6) we find that $\log K_{Y(rw)}$ and $\log K_{Y(sw)}$ show similar dependence on Q_{YO} and that $\log K_{Y(sw)}$ shows the more marked dependence. In each case there are significant linear correlations between $\log K_Y$ and Q_{YO} (Table 1) summarised by the equation,

$$\log K_Y = a_1 Q_{YO} + b_1 \quad (3)$$

The smaller number of data points and greater scatter of the river water plot (Fig. 3) reflect the greater difficulty of defining and quantifying a meaningful average for river water compared

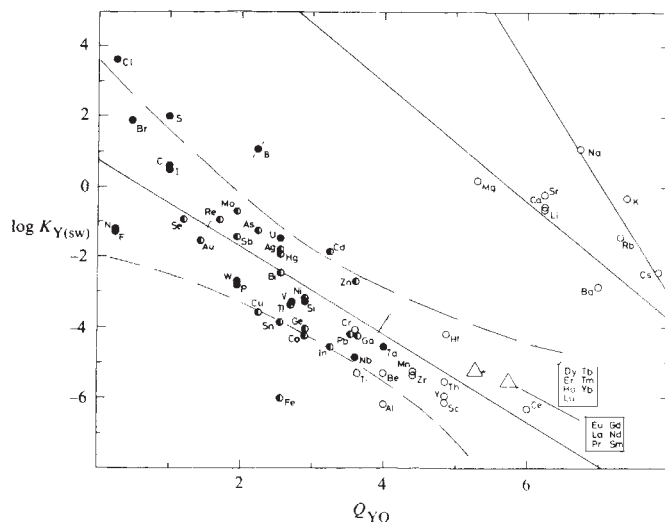


Fig. 2 The relationship between the seawater-crustal rock partition function ($K_{Y(sw)}$) and the electrostatic contribution to the Y-O bond energy (Q_{YO} , equation (7)). Classification (Table 2): $\circ$, L1 lithophiles; $\bullet$, chalcophiles and siderophiles; $\blacksquare$, L2 and L3 lithophiles. The solid lines indicate the linear regression fits (Table 1) and the dashed lines the Working-Hotelling confidence band at the 0.1% significance level¹⁹. Lines cutting across the main sequence of elements break the sequence into groupings discussed in the text. The oxidation states used are those considered to be thermodynamically stable in oxygenated seawater¹³ with the exception of Cr(III) and Mn(II) as there is evidence that these metastable forms are important in seawater. Thallium has been considered as Tl(III) because recalculation of the available data shows this to be the thermodynamically favoured oxidation state.

Table 2 Solid-state classification of the elements*

Geochemical classification	Preferential occurrence	Chemical characteristics	Q_{YO} range
Siderophile (S)	Metallic phase with Fe	More noble than Fe. Weak O and S affinity	1-4
Chalcophile (C)	Sulphide phase	Strong S affinity	1-4
		Cationic (L1)	>3.5
		Metal oxyanions	
		or oxocations (L2)	2-4
Lithophile (L)	Silicate phase	Strong O affinity	
		Non-metal oxyanions	
		or halide ions (L3)	<3†

* Adapted from the geochemical classification given in ref. 35.

† For halide ions $Q_{YO} \leq 1$.

with seawater. The anomalous positions of Ag and Sb in this plot probably arise from an overestimate of $\bar{Y}_B$ in each case particularly as the oceanic residence times of the elements calculated using river inputs are also anomalous (Fig. 1). This plot should be reconsidered when more accurate data become available for the global mean composition of river water. It should also be possible to construct analogous plots for particular fresh water systems (rivers or lakes) provided that the mean composition of the rocks in the appropriate drainage basins are used instead of $\bar{Y}_A$.

In the seawater plot (Fig. 2) we find that three groups of elements fall outside the main correlation. The elements Cl, S, B are normally considered to be 'excess volatiles'²⁹, that is they have a significant source in volcanic emissions which could result in an enhanced partition coefficient. Cd is fairly volatile (boiling point 765 °C) and may be enriched in the oceans in a similar way. In river water (Fig. 3) where the residence time is too short (<1 yr) for 'excess volatile' inputs to be significant these elements all fall on the main correlation. The other divergences from the main linear correlation are related to hydroxide solubilities. To achieve the expected value of $K_{Y(sw)}$, Fe would need to exceed its hydroxide solubility product by several orders of magnitude²¹. Iron therefore falls below the main correlation because of a combination of high abundance in crustal rock and low solubility in seawater. Although the data are more equivocal, a similar explanation probably applies to Ti and Al which are also very abundant in the Earth's crust³⁰. In river water (Fig. 3) where the lower pH and lower ionic strength make hydroxide precipitation less favourable these three elements also fall in the main correlation. The final group of anomalous elements are the Group IA and IIA metals (less beryllium). These elements differ from the rest of the electropositive elements in that they form soluble oxides and hydroxides. This arises from the relatively low charge/radius ratio of these cations, and as a result they show only a weak affinity for oxide lattices in aqueous media despite their low electronegativity. The extent to which the Group IA cations are extracted, for example, depends largely on the hydration energies that are released when the cations are incorporated into the water structure so that their $K_{Y(sw)}$ -values follow the Hoffmeister affinity sequence for ion-exchange³¹. These elements are only slowly removed from seawater so that they have long residence times (Fig. 1), and anomalously high K_Y values (Fig. 2). In river water (Fig. 3) where the timescale is much shorter the anomaly is much less marked.

The elements on the main correlation lines of Figs 2 and 3 are arranged according to their ionic affinity for oxygen, and the resulting sequence correlates well with the approximate geochemical classification of the elements first introduced by Goldschmidt³² (see Table 2). The lithophile/chalcophile division is a solid-state analogue of Ahrlund's classification of (a) and (b) cations in aqueous solutions^{33,34}: (a) cations prefer F, O, N as ligands while (b) cations prefer Cl, S, P. Both the solution and solid-state classifications show coherent groupings in the periodic table^{34,35}, but only the solid-state (geochemical) classification shows a clear correlation with the sequence of elements in Figs 2 and 3. We will redefine C, N, P, S as lithophiles as they occur as oxyanions in seawater. The litho-

philes can then be divided into three groups according to the nature of their interaction with oxygen (Table 2). The main correlation in Figs 2 and 3 has been divided into three parts. The middle portion contains virtually all the siderophiles and chalcophiles, which interact only weakly with oxygen. The ionic (L1, Table 2) lithophiles are grouped on the right of the main correlation (high Q_{YO} values) and the covalent (L3) lithophiles on the left (low Q_{YO} values). This clear correlation with the geochemical classification strongly suggests that solid-state chemistry controls the fractionation of the elements between the crustal rocks and seawater. Complexation of the elements in solution seems to be of lesser importance as the sequence of elements in Fig. 2 shows no correlation with the (a)/(b) classification of cations^{33,34}. In particular the typical (b) cations³⁴ (Ag, Au, Hg and Tl) which alone among the cations considered interact very strongly with chloride (the dominant ligand in seawater) show no anomalous behaviour. The solution chemistry of the elements is significant only where it directly affects hydroxide solubilities (Fe, Ti, Al and the Group IA and IIA metals).

The main correlation line in Fig. 2 can be used to predict $\log K_{Y(sw)}$ for the stable elements which have not yet been determined in seawater, that is Te and the platinum metals (Ru, Rh, Pd, Os, Ir, Pt). Using tabulated electronegativity values (ref. 16, p 73) we find $\log K_Y = -1.4$ for the platinum metals and -1.7

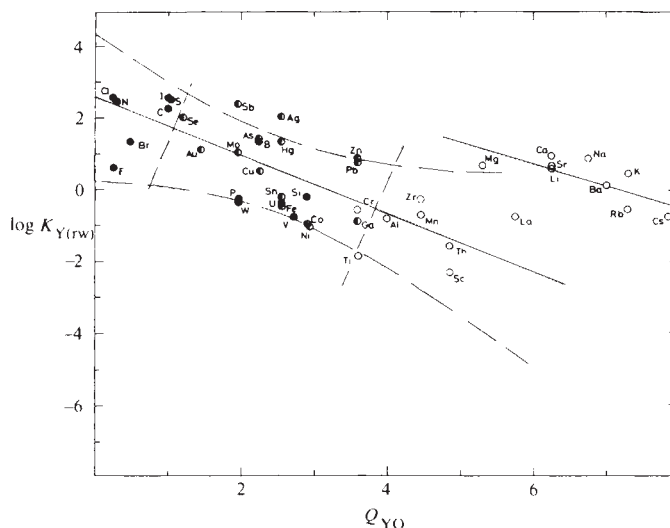


Fig. 3 The relationship between the river water-crustal rock partition function ($K_{Y(rw)}$) and the electrostatic contribution to the Y-O bond energy (Q_{YO} , equation (7)). Symbols as in Fig. 2. The solid lines indicate the linear regression fits (Table 1) and the dashed lines indicate the Working-Hotelling confidence band at the 1% significance level¹⁹. Lines cutting across the main sequence of elements break the sequence into groupings discussed in the text.

for Te. The crustal rock analyses for these elements are too scattered¹⁶ for an estimate of dissolved seawater concentration to be derived from K_Y . As expected Te (chalcophile) and the platinum metals (siderophiles) fall in the middle portion of the plot.

Weathering model revisited

We will now use a plot (Fig. 4) of the average seawater concentrations of the elements ($\bar{y}_C$, mol l⁻¹) against their average river water concentrations ($\bar{y}_B$, mol l⁻¹) to look again at the overall consequences of the weathering process. During the rapid passage through the fresh water reservoir, material is dissolved from the crustal rocks into water with a relatively low pH and ionic strength. As most of the elements are grouped within two orders of magnitude of $\log K_{Y(rw)} = 0$ in Fig. 3, the composition of the solids dissolved in the fresh water reservoir bears a reasonable resemblance to that of the parent rocks. As this water runs into the ocean bucket it enters a reservoir with a much longer holding time containing water which has (after many turns of the weathering cycle) a relatively high pH and ionic strength. Here material can accumulate up to the age limit

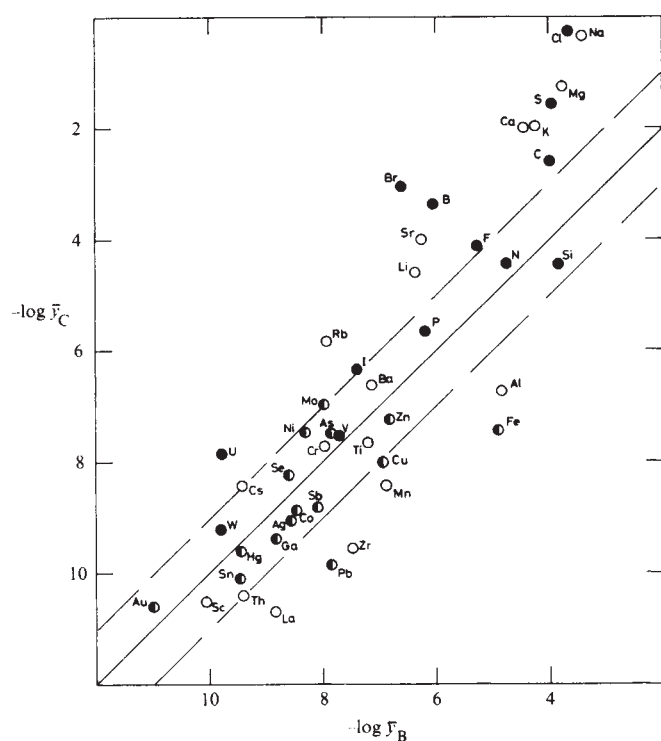


Fig. 4 The relationship between the mean concentrations of the elements in seawater ($\bar{y}_C$, mol l⁻¹)¹³ and in river water ($\bar{y}_B$, mol l⁻¹)¹³. To convert $\log \bar{y}_N$ to $\log \bar{y}_B$ subtract 4.43 units and 1.45 units respectively for river water and seawater. The solid line follows the 1:1 correlation and the dashed lines encompass points that fall within an order of magnitude of this line. Symbols as in Fig. 2.

of the reservoir itself unless its concentration is controlled at some intermediate level by interactions with the suspended matter. The residence times of bromine and chlorine (which are concentrated to the greatest extent in the oceans relative to the rivers) are approximately equal to the age of the ocean basins themselves (Fig. 1). The elements which accumulate in the oceans (Fig. 4, elements more than an order of magnitude above the 1:1 correlation) generally show significant positive deviations from the main sequence of elements in Fig. 2 indicating

either that their concentrations are controlled by their solution chemistry rather than by their solid state chemistry (Group IA and IIA elements) or that they might have a significant auxiliary source within the oceans (Cl, S, B).

More than half of the elements are already within an order of magnitude of their final oceanic concentration (Fig. 4) before they enter the ocean bucket. The bulk of these elements fall within the main correlation bands for both seawater (Fig. 2) and river water (Fig. 3). This suggests that the controlling processes are the same in both river water and seawater and are in geological terms kinetically fast.

All the elements that show a significant depletion in the oceans relative to the river water reservoir (Fig. 4; elements more than an order of magnitude below the 1:1 correlation) have oceanic residence times that are appreciably less than the time required for a single stirring revolution of the oceans (t_R , Fig. 1). In river water all of these elements fall within the main sequence of elements (Fig. 3) suggesting that, in the initial stages at least, their concentrations are controlled by their solid-state chemistry as outlined earlier. In seawater only iron and aluminium out of this set of elements fall significantly below the main sequence of elements (Fig. 2) and their oceanic concentrations are probably controlled by the solubility of their hydroxides. The other elements (La, Pb, Zr) fall clearly within the main sequence in Fig. 2 suggesting that in the oceans, too, their concentrations are controlled by their solid-state chemistry.

The non-equilibrium behaviour of nitrogen in the oceans was mentioned in connection with Fig. 1. Comparing Figs 2 and 3 we see that in Fig. 3 (river water) nitrogen is very close to chlorine, but that in Fig. 2 nitrogen falls almost five orders of magnitude below chlorine on the $\log K_Y$ axis. If, however, we include the elemental nitrogen present in the atmosphere and dissolved in seawater (that is the nitrogen which has been lost from the ocean reservoir to the atmosphere reservoir²¹), the value of $\log K_{N(sw)}$ falls only a short way below the value for $\log K_{Cl(sw)}$.

Conclusions

By considering that the oceans are in a steady state with respect to the flux of weathered components from the crustal rocks and that the whole weathering cycle has been processed many times during the Earth's history, we can use the partition function $K_{Y(sw)}$ to rationalise the observed composition of seawater. There is a strong correlation between $K_{Y(sw)}$ and the mean oceanic residence time ($\bar{t}_Y$) as both these parameters are controlled by the ease with which the various elements are exchanged between the rocks and the aqueous phase. The ionic contribution to the Y-O bond exerts an overall control over the partitioning of the elements between the aqueous phase and the solid phase and the correlation between $\log K_{Y(sw)}$ and Q_{YO} provides a rational explanation, in order of magnitude terms at least, for the seawater recipe. A similar correlation also exists for world average river water. The combination of these correlations provide a quantitative and general basis for Forchhammer's postulate¹⁰ and interrelate the concepts of reactivity and crustal abundance that have been frequently proposed as controlling factors in determining the chemistry of seawater^{13,22,36}.

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Transcription and processing of cloned yeast tyrosine tRNA genes microinjected into frog oocytes

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Xenopus oocytes transcribe cloned yeast tyrosine tRNA genes and process the RNA. The processing includes base modifications, addition of the CCA end, and splicing of the intervening sequence. The primary transcript has a 5' leader extension which varies considerably in sequence and in length between different tRNA^{Tyr} loci, but is nevertheless present in all of them.

METHODOLOGICAL advances have made possible the cloning and growth in *Escherichia coli* of segments of DNA coding for various individual eukaryotic genes and these genes can be sequenced using the rapid gel methods that have greatly simplified nucleotide sequence determination. However, the function of these nucleotide sequences is not always readily apparent from the primary structure. For example, it is difficult to recognise from the DNA sequence the location of promoters and the precise start of the primary transcript. Thus there is a need for a functional assay for cloned eukaryotic genes. Here, we analyse the expression of cloned yeast tRNA^{Tyr} genes after microinjection into living *Xenopus laevis* oocytes.

Saccharomyces cerevisiae has eight unlinked tRNA^{Tyr} loci¹, each of which can be mutated into an ochre, amber or UGA suppressor^{2,3}. All eight genes code for the same mature tRNA^{Tyr} sequence. Seven of these genes have been cloned⁴ and four have been sequenced (ref. 5 and Phillipsen, Cameron and Davis, personal communication). An intervening sequence of 14 base pairs was found inserted at the 3' end of the anticodon. The sequence of the intervening sequence is also conserved between the various genes, with the exception of one base pair which has been observed to vary. However, at the flanking region at the 5' (starting) side of the tRNA sequence, the DNA sequences are almost totally dissimilar between the different genes⁵.

The intervening sequence is normally transcribed in yeast, then excised and the RNA molecule re-ligated to produce the mature tRNA^{6,7} (a process hereafter referred to as 'splicing'). This is known from the existence of a yeast temperature-sensitive mutant⁸ that accumulates a 92-nucleotide tRNA^{Tyr} precursor with the same 5' and 3' ends as the mature tRNA and

containing the intervening sequence. This work did not indicate whether this precursor represented the primary transcript, or if there is an even longer initial transcript (see below).

DNA microinjected into the nucleus of *Xenopus* oocytes is transcribed⁹ and translated¹⁰. Genes transcribed by RNA polymerase III are expressed very efficiently and with high fidelity in oocytes, as shown initially for 5S ribosomal RNA genes^{11,12} and subsequently for tRNA genes^{13–15}. Recently, the transcription of tRNA genes from *Drosophila* and the fission yeast *Schizosaccharomyces pombe* has also been reported¹⁷ in a cell-free system derived from *Xenopus* oocytes¹⁶.

In this study we use plasmids containing yeast tRNA^{Tyr} genes for a detailed analysis of the primary transcript of a eukaryotic tRNA gene, and its processing in living cells. Our analysis was greatly facilitated by the use of genes of known nucleotide sequence, and of several members of the same gene family. We find that the initial transcript contains, in addition to the intervening sequence, an extra segment at the 5' end. This 5' leader sequence is not conserved, and varies considerably in length and nucleotide sequence between the various members of the tRNA^{Tyr} gene family. In addition, we show that many of the frog enzymes involved in tRNA production are not species specific, as they can accurately recognise yeast genes. These enzymes include RNA polymerase III, RNase P, RNA methylases, tRNA nucleotidyl transferase (CCase) and the splicing enzyme(s).

Frog oocytes transcribe yeast tRNA genes

Figure 1 shows the result of an experiment in which oocytes were injected into the nucleus with DNA of plasmid pYT-C and [α -³²P]GTP. Plasmid pTY-C (ref. 5) contains a 3-kilobase fragment of yeast DNA that includes a tRNA^{Tyr} gene and its flanking sequences cloned in the plasmid pBR 322 (ref. 18). This tRNA^{Tyr} gene corresponds to the known nonsense suppressor locus SUP 8 (M.V.O., Longhey and Hall, in preparation). Several newly synthesised RNA bands are detectable after injection of the plasmid, and their approximate lengths can be estimated using appropriate RNA markers (Fig. 1, lanes f, j). One faint band of 108 nucleotides and three main bands of 104, 92 and 78 nucleotides can be distinguished.

Thirty minutes after injection (Fig. 1, lane *b*) only the 108 and 104 nucleotide bands are detectable. The weak 108 band is always found, and although not seen very clearly in Fig. 1, is clearly visible in Fig. 4, lanes *a* and *b*, and in Fig. 5, lanes *a* and *b*. At longer incubation times the 92-nucleotide precursor is the most abundant product (Fig. 1, lanes *c-e*). The 78-nucleotide band co-migrates with purified yeast tyrosine tRNA (compare

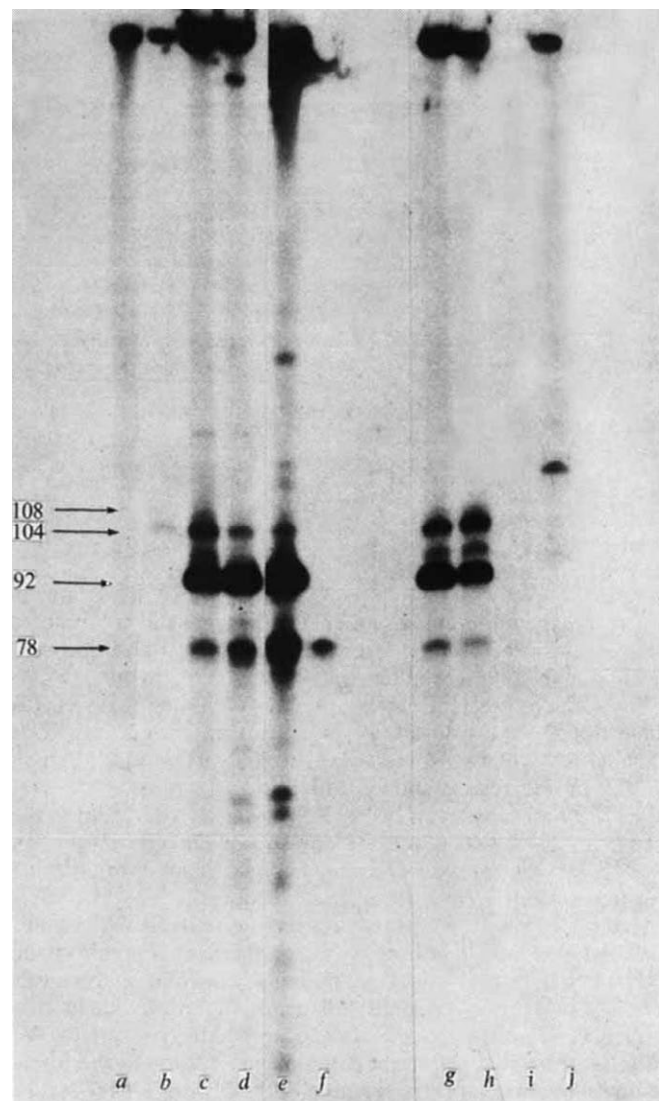


Fig. 1 Autoradiogram of ^{32}P -RNAs synthesised by *Xenopus* oocytes injected into the nucleus with a plasmid containing a yeast tRNA^{Tyr} gene and cultured in saline solution for various times³⁶. Each oocyte was injected with 30 nl of a solution containing 400 $\mu\text{g ml}^{-1}$ pYT-C DNA and 10 mCi ml^{-1} [α - ^{32}P]GTP (350 Ci mmol^{-1} , Amersham). After incubation the RNA was extracted³⁷ and electrophoresed in a 7 M urea-12% polyacrylamide gel using thin (0.35 mm) spacers³⁸. About 1 oocyte worth of RNA was loaded into each slot, and the gel exposed for 2 d using an Ilford fast tungstate intensifying screen and pre-exposed Fuji Rx film³⁹. Samples *e* and *f* were run in adjacent lanes in a different 12% gel. Lane *a*, oocytes injected with pBR 322 DNA and incubated for 6 h (no yeast DNA); *b*, pYT-C injected and incubated for 30 min; *c*, pYT-C, 6 h; *d*, pYT-C, 12 h; *e*, pYT-C, 24 h. Lane *f*, purified yeast tyrosine tRNA marker¹, which was end-labelled with polynucleotide kinase and [γ - ^{32}P]ATP⁴⁰. Lane *g*, oocytes injected with pYT-C and incubated for 6 h; *h*, same as *g* but injected with 1 $\mu\text{g ml}^{-1}$ α -amanitin; *i*, same as *g* but with 100 $\mu\text{g ml}^{-1}$ α -amanitin. Lane *j*, oocytes injected with pXlo-8, a plasmid containing *Xenopus* 5S genes¹² included here as a size marker (the 5S band has 120 nucleotides, and the pre-5S one is 135 nucleotides long).

lanes *e* and *f*, Fig. 1). The proportion of the 78-nucleotide material increases with time, but is never more than the amount in the 92-precursor band (lanes *c*, *d*, *e* in Fig. 1 show 6, 12 and 24 h incubations, respectively). Even after 4 d in culture (data not shown), the 78 band represents only 30% of the RNA induced by the injection of yeast DNA. (Note, however, that oocytes transcribe the injected DNA continuously throughout this period.)

In addition to these bands, other minor products are detectable. A minor band of about 97 nucleotides is consistently observed (Fig. 1, lane *g*), and probably represents a true intermediate step in the processing of the longer initial transcript into the 92 band, as it is quite prominent after 2 h of incubation (Fig. 4, lane *a*).

Yeast tRNA^{Tyr} genes are transcribed by the oocyte RNA polymerase III, as shown by α -amanitin inhibition (Fig. 1, lanes *g*, *h*, *i*). α -Amanitin, 1 $\mu\text{g ml}^{-1}$, injected with the DNA did not significantly affect tRNA transcription. At this concentration α -amanitin abolishes RNA polymerase II transcription in oocytes injected with SV40 DNA^{10,12} or prokaryotic plasmids.¹⁹

We conclude that yeast tRNA^{Tyr} genes are transcribed in oocytes by the correct RNA polymerase (type III). We can detect a weak band 108 nucleotides long, which is then presumably processed into three main RNAs of about 104, 92 and 78 nucleotides.

Initial transcripts have a 5' leader extension

Knowledge of the DNA sequence (Fig. 2) has greatly facilitated analysis of the composition of the RNA bands; this was done using the classical RNA sequencing techniques of Sanger *et al.*^{20,21}

As the main band accumulated is about 92 nucleotides long, it seemed possible that it could be the same kind of precursor as that accumulated in the yeast mutant^{6,7}, which has the same ends as the mature tRNA but contains the intervening sequence (tRNA^{Tyr} has 78 and the intervening sequence 14 nucleotides; 78 + 14 = 92). Figure 2 shows the ribonuclease T1 oligonucleotides that can be predicted for such a molecule (this enzyme cuts RNA after G residues). Analysis of the T1 fingerprint shown in Fig. 3, left panel, confirmed that the 92-nucleotide material synthesised in oocytes contains the intervening sequence.

The spots in this GTP-labelled fingerprint were identified by elution and secondary digestion with pancreatic ribonuclease (which cuts after C and U). As the oocyte does not exchange the ^{32}P label of one injected labelled nucleotide into other nucleotides¹⁵, the spots can be recognised by the nearest neighbour to the G residue (underlined in Fig. 3, central panel). For example, the T1 oligonucleotide ApCpU^{*}Gp would be cut by pancreatic ribonuclease into ApCp/U^{*}Gp, giving U as the nearest neighbour to G. The identifications were confirmed by fingerprints of ATP or CTP labelled materials, in which certain spots are not labelled and others vary in intensity according to the number of labelled phosphates present. For the purposes of this study the most important spots are numbers 15 and 16, which contain the intervening sequence (and which will disappear in the 78 material), and spot 14, which contains the 5' end of the molecule.

Figure 3, right panel, shows the T1 fingerprint of the 104-nucleotide band. The oligonucleotides of this precursor are the same as for the 92 material, except that spot 14 (which was the 5' end of this RNA) no longer appears in its usual position. Two new large oligonucleotides appear (hatched tracing in centre panel, Fig. 3), and the nearest-neighbour data (Fig. 3) are consistent with their being UUAUUG and ACUCUCG, which originate from the region of the pYT-C DNA at the 5' side of the mature tRNA.

We conclude from this that the larger precursor differs from the 92-nucleotide band at its 5' end, having a 5' leader whose sequence is consistent with that predicted from the DNA sequence of the gene.

-20 -10 -1

pYT-A ----- CTTTCTTCAACAATTAAGTA
GAAAGAAGTTGTGTAATTTCAT

pSU4-A ----- CTTTCTTCAACAATTAATAA
GAAAGAAGTTGTGTAATTTCAT

pTY-G ----- CGTGTTTTACAACAAAAACCA
GCACAAAATGTTGTTTTCGCT

↓

pYT-C ----- TGATGGTTATCAGTGAATTGA
ACTACCAATAGTCAATTAAGT

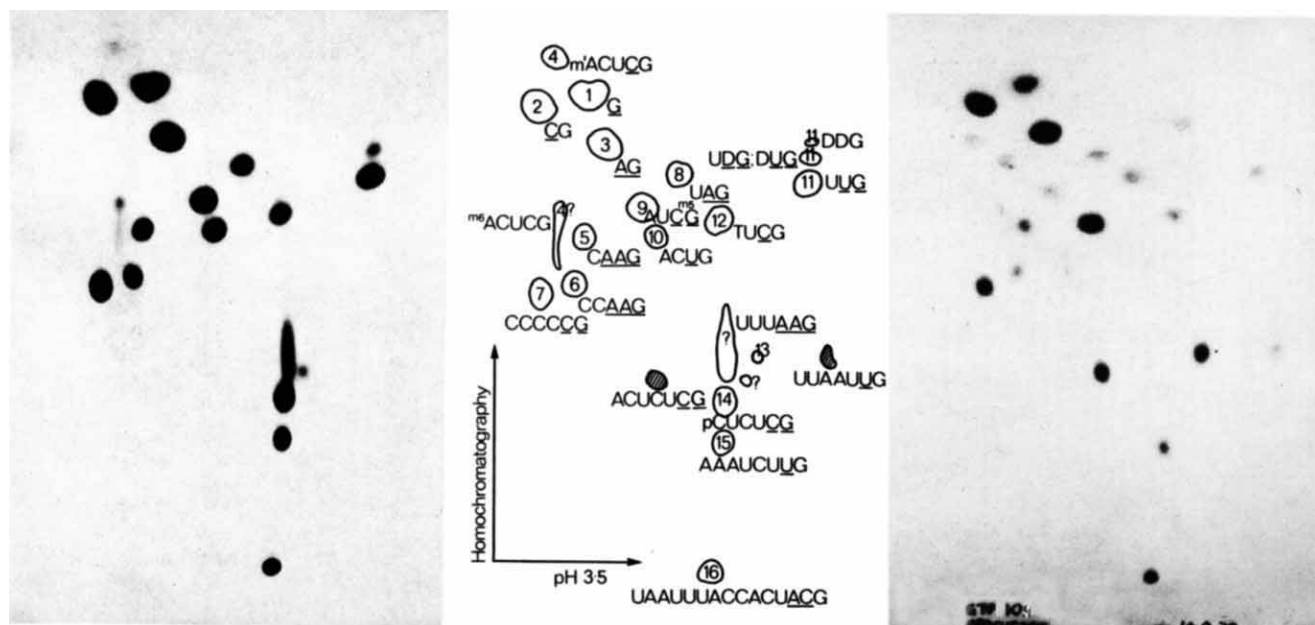
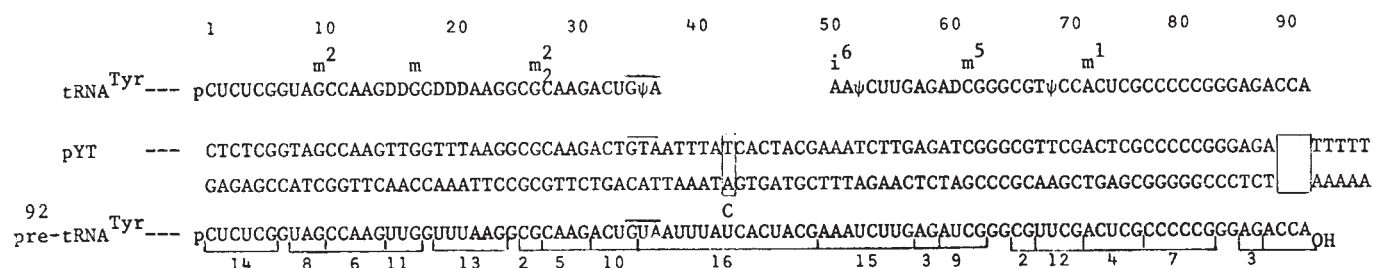


Fig. 3 Fingerprints of ribonuclease T1 oligonucleotides of tRNA^{Tyr} precursors synthesised by oocytes injected with pYT-C and [α -³²P]GTP. Left panel, 92-nucleotide precursor; right panel, 104-nucleotide precursor; centre panel, tracings indicating the composition of each spot, with spots numbered as in Fig. 2; the oligonucleotides that were found to be labelled after nearest-neighbour analysis (secondary digestion with pancreatic RNase) are underlined. The two new long oligonucleotides that appear in the 104 material are hatched. The 104-nucleotide material has some short oligonucleotides (paler spots on the upper part of the fingerprint) which come from the background oocyte RNA. The background of endogenous transcripts is not negligible in this case because the 104 band is relatively weak, and the fingerprint has to be exposed for longer periods. We cannot exclude the possibility that the 104 band may contain an additional short oligonucleotide, because it could be masked under one of these background spots. The RNA bands were eluted⁴⁰ from preparative gels and then digested, fingerprinted and further analysed as described by Barrell²¹. The pancreatic RNase secondary digestion products were analysed by electrophoresis in 40 cm DEAE-cellulose thin layer plates (Cel. 300 DEAE, Polygram, Macherley-Nagel) at pH 3.5 (1,000 V for 3½ h). The oligonucleotides migrate in the same way as in the usual DEAE-paper electrophoresis method²¹, but we find that thin layers are easier to handle.

Mapping the primary transcript

The primary transcript is the weak 108-nucleotide band. This was established by injecting ATP or GTP labelled in the γ -phosphate. As shown in Fig. 4, $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ was incorporated only into the 108 band, whereas all other bands lack the terminal triphosphate. According to the length of this molecule, this should start at about position -19 . (There is evidence that the large precursors do not have the CCA end (see below) but a short row of U residues at the 3' end of the primary transcript would have gone unnoticed in our fingerprints.) The only A residue in this region is in position -19 of pYT-C (Fig. 2), which is therefore the most likely starting place.

The presence of this 5' leader extension was confirmed by comparing the transcription of tRNA^{Tyr} genes from different loci. We mentioned earlier that the sequence of the region flanking the 5' side of the tRNA sequence varies considerably among the different yeast tRNA^{Tyr} loci. From the work described in the previous section, we now know that this region is transcribed into RNA in the initial transcripts. The plasmids pYT-C and pYT-A, for example, differ in the position of all their

A residues (except for the one at position -5) in this region (Fig. 2). Therefore, the initial transcripts of these two genes should be of different lengths, and Fig. 5 shows that this is indeed the case.

Thirty minutes after injection, pYT-C induces the 108 and 104 bands (Fig. 5, lane a), whereas the equivalent bands induced by pYT-A are about 5 to 6 nucleotides shorter (Fig. 5, lane c). Assuming that transcription starts with A or G, the most likely start for the primary transcript of pYT-A is in one of the A nucleotides in positions -12 or -11 (Fig. 2). The plasmid pSU4-A, which contains an *ochre* suppressor mutant of gene A, induces the same bands as pYT-A (Fig. 5, lanes e, f), as expected from its DNA sequence (Fig. 2). Plasmid pYT-G (Fig. 5, lanes g, h) produces several bands, the longest of which has about the same length (or, in some gels, slightly more) as in pYT-A and

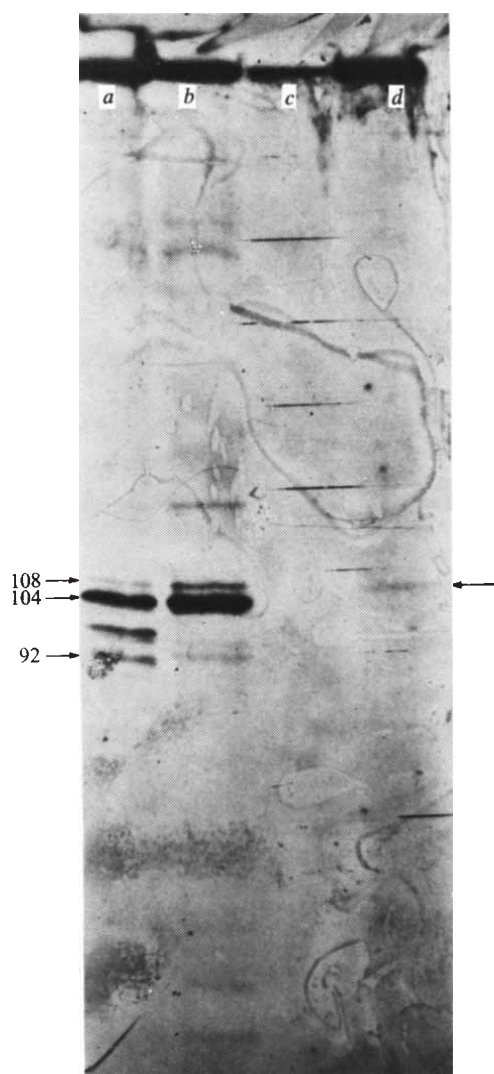


Fig. 4 The 108 band is the primary transcript. Gel electrophoresis of the RNAs synthesised in *Xenopus* oocytes injected with pYT-C DNA and with: lane a, $[\alpha\text{-}^{32}\text{P}]\text{GTP}$, 2 h incubation; b, $[\alpha\text{-}^{32}\text{P}]\text{GTP}$, 30 min incubation; c, $[\gamma\text{-}^{32}\text{P}]\text{GTP}$, 2 h incubation; d, $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, 2 h incubation. The position of the various precursor bands is indicated. The γ -labelled nucleotides were injected at 50 mCi ml^{-1} . The band of the primary transcript is indicated in slot d.

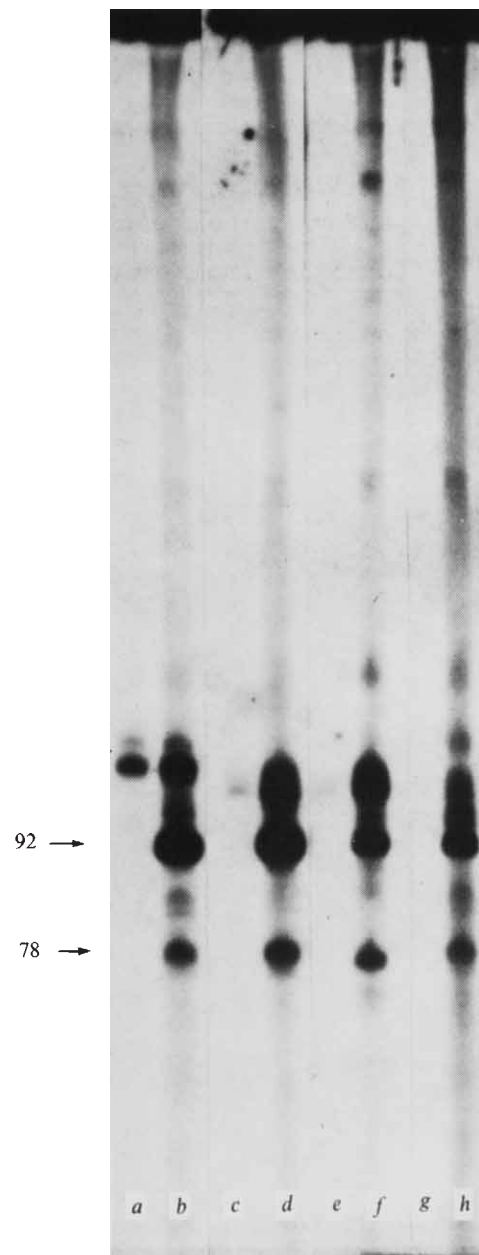


Fig. 5 The initial transcripts vary in length in different tRNA^{Tyr} loci. Gel electrophoresis of RNAs synthesised by oocytes injected with $[\alpha\text{-}^{32}\text{P}]\text{GTP}$ and: lane a, pYT-C DNA, 30 min incubation; b, pYT-C, 6 h incubation; c, pYT-A, 30 min incubation; d, pYT-A, 6 h incubation; e, pSU4-A, 30 min incubation; f, pSU4-A, 6 h incubation; g, pYT-G, 30 min incubation; h, pYT-G, 6 h incubation.

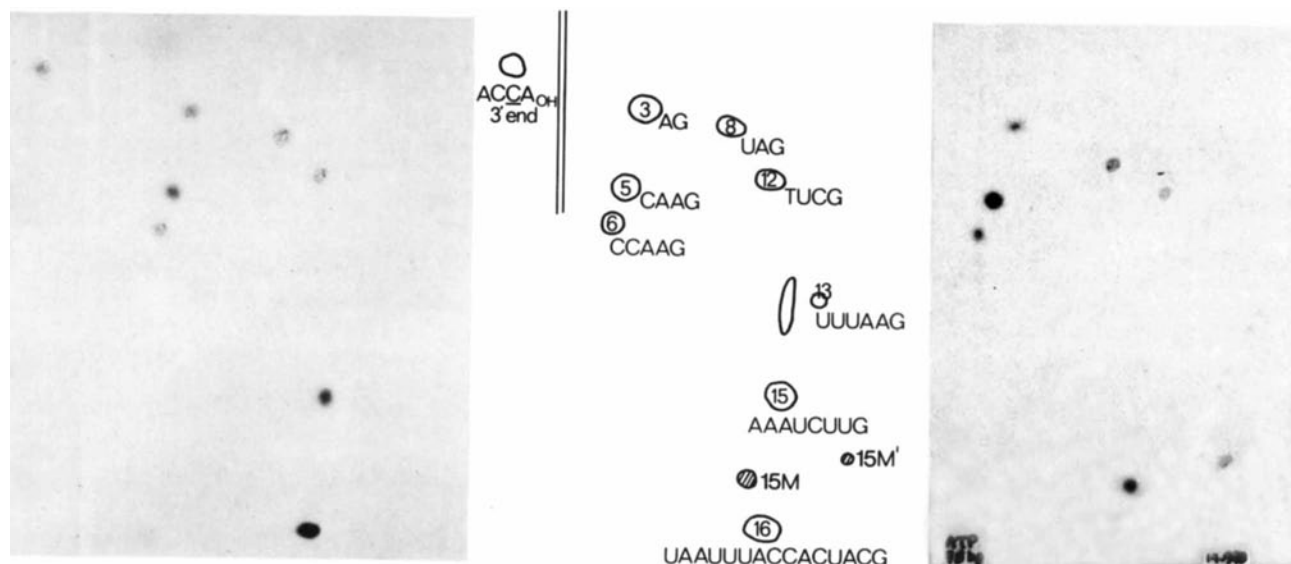


Fig. 6 T1 oligonucleotides of the 92-nucleotide material (left panel) and the 78 nucleotide band (right panel) induced by the injection of pYT-C DNA into *Xenopus* oocytes. Separation was as in Fig. 3. The oocytes were labelled with [α - 32 P]ATP, and for this reason many of the spots present in Fig. 3 are unlabelled here. Spot 12 does not contain A but is still labelled because in the RNA its G residue has an A as nearest neighbour (sequence TψCGA). The CCA end oligonucleotide runs very slowly in the first dimension, and was therefore run in a separate second dimension plate. Spots 15 and 16 are absent in the 78-nucleotide band, and a new spot (15 M, hatched symbols) appears.

pSU4-A. All four plasmids produce the same 92 and 78 nucleotide bands at longer incubation times (Fig. 5, lanes *b, d, f, h*); this is as expected, as the four genes have a very similar nucleotide sequence within this region (Fig. 2).

We conclude that the primary transcript of pYT-C starts in pppA, and that it has a 5' leader sequence most probably 19 nucleotides long. We believe this to be the first time that the transcription initiation site of an eukaryotic tRNA gene has been mapped within a region of known DNA sequence. Frog oocytes injected with DNA and γ - 32 P-triphosphates should be useful for identifying primary transcripts in other cloned genes.

Base modifications and the CCA end are added post-transcriptionally

Oocyte enzymes can modify at least some bases of tRNA^{Tyr} transcripts correctly. For example, of the six labelled cytosines that we examined in the nearest-neighbour analysis of the fingerprint shown in Fig. 3, left panel (92-nucleotide band labelled with GTP), only one cytosine was found to be methylated. The 5-methylcytosine was found in oligonucleotide 9 (data not shown) and corresponds to the only cytosine normally methylated in yeast tyrosine tRNA^{22,23}. Other bases are probably also modified as judged from mobility changes in the fingerprints (for example, spots 4 and 11 in Fig. 3, left panel), but they were not studied in detail.

The various processing steps occur in a precise order. For example, oligonucleotide 9 is completely modified by the 92-precursor stage, but it is not modified in the 104 band. As seen in Fig. 3, spot 9 (AUCG) is displaced in the 104 fingerprint, where it co-migrates (confirmed by nearest-neighbour analysis) with spot 10 (ACUG). This is expected if spot 9 is not modified, because oligonucleotides 9 and 10 have the same length and base composition (one of each of the four nucleotides), and therefore the two spots should not be resolved by the fingerprinting technique in their unmodified form. (In yeast tRNA oligonucleotide 9 is modified into AD^mCG; and the dihydrouridine is the modification most likely to produce such a displacement in the fingerprint.)

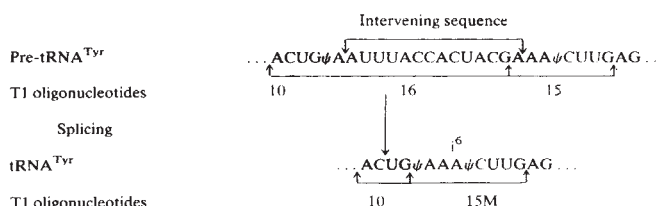
The CCA 3' end of the tRNA^{Tyr} is not encoded in the DNA, but is added post-transcriptionally by a tRNA nucleotidyl transferase (CCase). A spot corresponding to the oligonucleotide ApCpCpAOH was found in ATP (Fig. 6) and CTP labelled

fingerprints of the 78 and 92 nucleotide species, but not in GTP-labelled ones. The sequence of this spot was confirmed by nearest-neighbour analysis after pancreatic ribonuclease digestion (C with [α - 32 P]ATP and AC with [α - 32 P]CTP). A nucleotidyl transferase activity capable of adding a terminal A residue to tRNA and viral RNAs has been previously described in injected oocytes^{24,25}. The CCA end is present in the 92 and 78 nucleotide bands, but is absent from the 104 precursor (data not shown).

The intervening sequence is excised and the molecule re-ligated

The final product of the *Xenopus* oocyte processing seems to be a covalently intact 78-nucleotide species that does not contain the intervening sequence. Our data do not unequivocally demonstrate that the *Xenopus* splicing enzymes make the identical splice that occurs in yeast, but the splicing point can be located at or within two or three nucleotides of the correct site.

The 78 and 92 nucleotide bands differ only in the region of the intervening sequence. Figure 6 shows that T1 oligonucleotides 15 and 16 (which contain the intervening sequence) are absent in the 78 material, whereas all the other spots are conserved (this is known for GTP, ATP and CTP labelled fingerprints). In addition, a new spot appears in the tRNA-size material (15M in Fig. 6). In yeast, the splicing of the intervening sequence normally involves:



As in yeast, spot 15M made in oocytes is a larger derivative of oligonucleotide 15. This was identified by nearest-neighbour analysis of spot 15M which gave U using α GTP, and AAAU using α CTP. (There is also a second spot, much less intense, which appears reproducibly in the 78 material and is indicated as 15M' in Fig. 6; this minor component is probably also derived

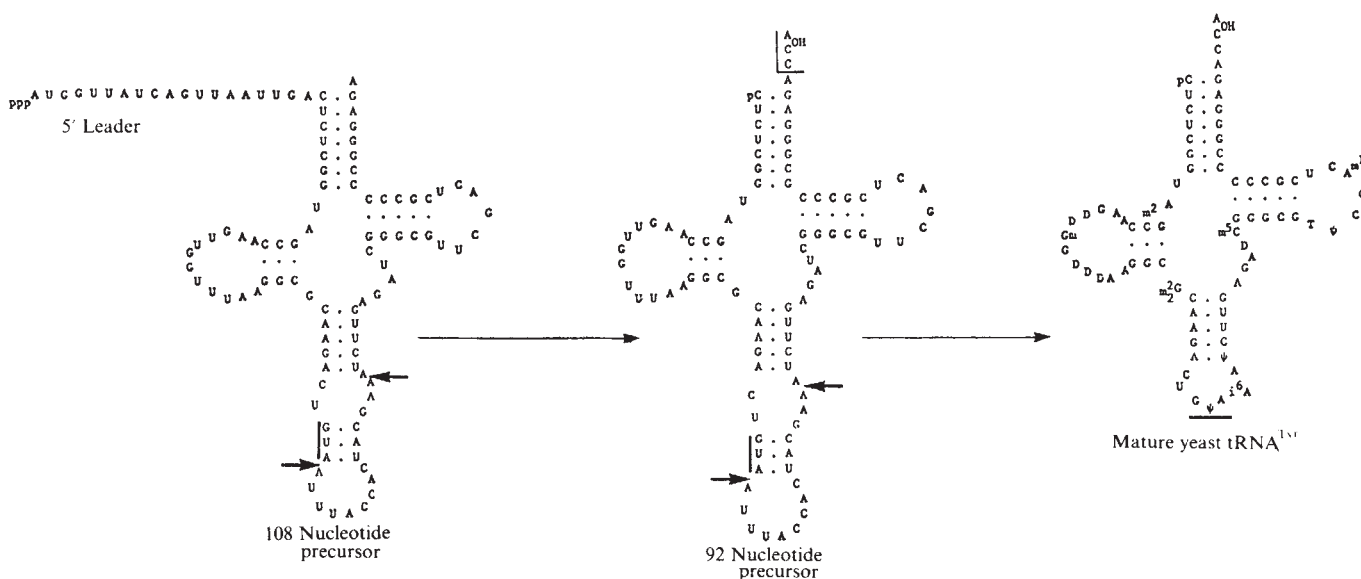


Fig. 7 Main processing stages in the expression of *Saccharomyces cerevisiae* tyrosine tRNA gene C. The primary transcript has a 5' leader sequence 19 nucleotides long, and an intervening sequence 14 nucleotides long. The 5' leader segment differs in length and composition in transcripts of different tRNA^{Tyr} genes. The 5' leader is removed in at least two steps and the molecule is converted into a 92-nucleotide precursor that has been previously identified in yeast^{6,7}. The 92-nucleotide molecule contains several base modifications (not shown here) and the CCA end that is added post-transcriptionally. The intervening sequence is then excised and the molecule re-ligated to produce mature tRNA^{Tyr} (shown here with the base modifications that occur in yeast^{22,23}). The position of the anticodon in the various precursors is underlined. The secondary structure shown here for the 92-nucleotide precursor is the one preferred in ref. 7.

from spot 15, because its nearest neighbour to G is U.) Spot 15M from oocytes, however, moves more slowly in the first dimension than does its yeast counterpart⁷. This mobility difference could be due to a difference in base modification (oligonucleotide 15M from yeast contains an isopentenyl adenosine). However, we cannot rule out the alternative explanation that oligonucleotide 15M has an anomalous mobility because the *Xenopus* processing system splices the yeast precursor incorrectly, although this is unlikely. The splicing site must not lie within oligonucleotide 10 because this spot is found in α -CTP and α -GTP labelled fingerprints of the 78-nucleotide material (data not shown). The nearest-neighbour analysis of spot 15M requires that this oligonucleotide contains the sequence AAAUC, and UG in its 3' end. These data, nonetheless, do not exclude the possibility of a splice giving an oligonucleotide 15M at least 3 nucleotides longer than in yeast (for example, UAAUAAUCUUG). We consider this possibility unlikely in view of the co-migration of the mature yeast tRNA^{Tyr} and the oocyte product in polyacrylamide gels (Fig. 1).

After the intervening sequence is excised, the molecule is re-ligated. This is known because the 78 band maintains the same mobility after heating the RNA at 95 °C for 3 min in 2 volumes of formamide before loading directly on an acrylamide-7 M urea gel. In these denaturing conditions²⁶ this band would have migrated as two half-molecules if not covalently closed. Splicing of yeast tRNAs by *Xenopus* cells has also been found for the tryptophan tRNA genes using *in vitro* nuclear extracts (Ogden, Beckmann, Abelson, Söll and Schmidt, manuscript in preparation).

Conclusions and prospects

Figure 7 outlines the sequence of processing steps for yeast tRNA^{Tyr} that we have inferred from the analysis of the major RNAs that accumulate in oocytes in response to the injection of DNA from the plasmid pYT-C. Rigorous demonstration of precursor-product relationships by means of pulse-chase experiments is not feasible in this experimental system, but the time course with which the different RNA species accumulate is consistent with the view that mature tRNA^{Tyr} is formed by an orderly series of steps in which the RNAs represented in Fig. 7 are sequentially formed. In this interpretation, the 5' leader is

removed in at least two stages, resulting in a molecule with the same 5' end as the mature tRNA, but still containing the intervening sequence. Several base modifications and the addition of the CCA end are detectable for the first time at this stage. This 92-nucleotide precursor has been identified *in vivo* in a yeast mutant^{6,7}, but none of the larger RNAs has been previously described. Yeast therefore has tRNA precursors with 5' leader extensions similar to those described in bacteria²⁷.

The possible significance of the 5' leader sequence is intriguing. This transcribed region not only varies considerably in nucleotide sequence⁵ between the different tRNA^{Tyr} loci, but also in length (Fig. 5). However, all four tRNA^{Tyr} genes examined so far have a 5' leader sequence. One is then left with the question of why these segments of RNA with no obvious sequence or length constraints are always present, and not eliminated altogether. Although this cannot be answered, one possibility is that they introduce order in the intricate processing that the primary transcript must undergo before becoming tRNA, that is, some modifications could occur while the 5' leader is still present, but others cannot occur until this segment is removed.

The final major processing step—the removal of the intervening sequence and re-ligation of the RNA—is not as efficient (30% maximum) in *Xenopus* oocytes as in wild-type yeast cells⁸, but in some ways it is remarkable that it is conserved at all in such a highly heterologous system. Clearly, the enzymes involved can tolerate extensive changes in primary sequence, as the yeast and *Xenopus* tRNA^{Tyr} genes have diverged markedly (S. Clarkson and F. Müller, personal communication).

The specificity with which *Xenopus* RNA polymerase III recognises these transcriptional units is particularly surprising in view of the major biochemical differences that have been observed between the yeast enzyme and those from higher eukaryotes. The yeast enzyme differs from vertebrate RNA polymerase III in both its α -amanitin insensitivity^{28–30} and subunit composition³¹. We have no direct evidence that the *Xenopus* enzyme initiates transcription at the same site as in yeast. The demonstration of transcription initiation in the immediate proximity of the tRNA coding sequence strongly suggests, however, that the *Xenopus* enzyme is correctly recognising the true yeast transcription unit. For example, the plasmid

pYT-C contains 7 kilobases of DNA, but the only discrete transcripts originate from the tRNA^{Tyr} gene region, which represents <2% of the plasmid. This also applies to the other plasmids used in this study. Our results suggest that although *Xenopus* and yeast polymerase III differ in their biochemical properties, they still recognise the same initiation signals in DNA.

More extensive DNA sequencing will be required in the region preceding the transcription initiation sites within the different tRNA^{Tyr} genes before any conclusions can be drawn about the nature of polymerase III 'promoter' sequences³². The yeast tRNA^{Tyr} gene system has the major advantage that it should be possible to identify base pairs essential to the initiation of transcription by obtaining mutants that affect this process. All eight yeast tRNA^{Tyr} genes correspond to known nonsense suppressor loci^{2,3} and the set of cloned restriction fragments that contain these genes has been cross-correlated with the set of genetic loci (M.V.O., Longhey and Hall, in preparation). Methods are also well established for obtaining second-site mutations within these genes by starting with a functional suppressor and selecting for the loss of suppression^{33,34}. The system described here should provide an effective technique for

screening mutant genes for defects in transcription and in the different processing steps.

The finding that *Xenopus* enzymes can carry out several key steps in the processing of yeast tRNA^{Tyr} transcripts supports the view that yeast suppressor genes might provide nonsense suppressors if introduced into the cells of higher organisms³⁵. However, we do not yet know whether a fully functional gene product is synthesised.

We hope that in the future some of the prospects outlined above might be achieved. In the meantime, however, it is already clear that very precise information about cloned genes and their primary transcripts can now be obtained using the most crude (but most physiological) assay system of all—their expression in an intact living cell.

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letters

Angular dimensions of accreting young stars

ALTHOUGH every star passes through a pre-main sequence stage during its formation, only a few astronomical objects have been considered as likely candidates for the early protostellar evolutionary stage¹. The bulk of the energy observed as radiation emitted by a compact source in the near IR, from 2 to 10 μm , is attributed to thermal emission from a dust shell, where the opacity is a rapidly varying function of the wavelength. To study radiative transfer models, the knowledge of the spatial intensity distribution on the object surface in the near IR is crucial. We have developed a method of IR speckle interferometry² which allows the spatial spectrum of an object in a given direction in the modulus sense to be determined up to the frequency cut-off of the telescope. As usual in speckle interferometry techniques the phase is lost unless sophisticated phase-restoration techniques are used. We applied this method

to several protostellar candidates: the BN object, W3IRS5, R MonIRS3, CRL2591 and S140IR at wavelengths 4.8 and 3.5 μm . We present here the results of dealing with BN and W3IRS5, and a more detailed interpretation of the whole set will be given elsewhere.

The specklograph has the following configuration²: the image is formed at the Coudé focus ($f/181$) of the Mayall 3.81-m telescope from Kitt Peak National Observatory. A scanning mirror driven by a saw-tooth wave generator displaces this image on a slit: the motion is perpendicular to the slit and linear with time. The slit is of the order of the seeing disk size, and its width smaller than the Airy radius. The flux is collected through a cold filter ($\lambda = 4.8$ or 3.45 μm ; see Table 1) onto an InSb detector. Alternate scans were made on the object and on adjacent sky to measure in similar conditions the object power and the system- and sky-noise power. One-dimensional power spectra of the source were obtained through the following process: (1) the power spectrum of the source and of the noise were averaged over a given integration time T_0 amounting to N_E

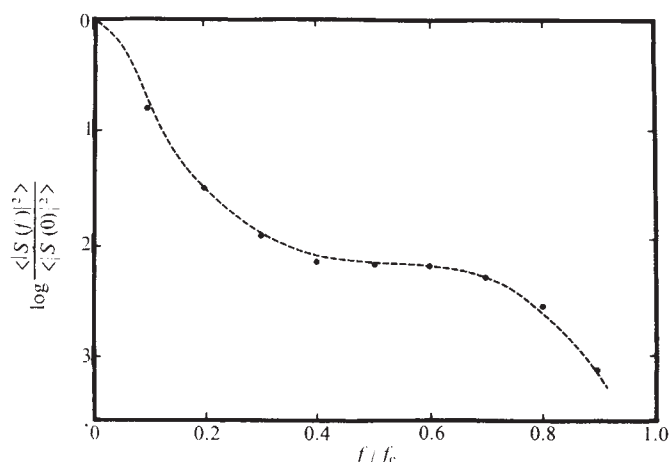


Fig. 1 The atmosphere and telescope normalised MTF, $\langle |S(f)|^2 \rangle / \langle |S(0)|^2 \rangle$ as measured at $4.8 \mu\text{m}$ on a point source (μGem). The observations (●) are compared to the best available model of atmospheric MTF (log normal model). The telescope MTF is the theoretical diffraction limited pupil. The resulting MTF is given by the dashed line, and the best fit with the data is obtained for $r_0(4.8 \mu\text{m}) = 78 \text{ cm}$.

scans with individual duration τ ; (2) the noise power spectrum was subtracted from the source power spectrum; (3) the resulting spectrum was divided by the atmosphere plus telescope modulation transfer function (MTF), in the modulus sense.

The latter MTF is obtained on a bright point-like star, the observation of which is alternated with the observation of the object. Figure 1 shows a typical MTF including the atmosphere and the telescope transfer functions, as determined on a point source star (μGem). A more detailed analysis of the MTF properties is given in refs 2, 3. We obtain the power spectrum data points from

$$\langle |S_\lambda(f)|^2 \rangle = \langle |i_\lambda(f)|^2 - |b_\lambda(f)|^2 \rangle_{\text{reference star}}$$

This process is summarised by

$$\text{estimation of } |o_\lambda(f)|^2 = \frac{\langle |i_\lambda(f)|^2 - |b_\lambda(f)|^2 \rangle_{\text{object}}}{\langle |i_\lambda(f)|^2 - |b_\lambda(f)|^2 \rangle_{\text{reference star}}}$$

for $f \leq f_c$, with $f_c = D/\lambda$ the frequency cut-off of the telescope. $|o_\lambda(f)|^2$ is a cross-section of the object two-dimensional power spectrum in the scan direction, versus spatial frequency f . $i_\lambda(f)$ and $b_\lambda(f)$ are respectively source and noise spectra. Averaging is made over the observation time. The estimated $|o_\lambda(f)|$ is an average over the band pass $\Delta\lambda$.

A phase-demodulation synchronised with the scan provided a real-time detection of the object and allowed fine guiding to an accuracy well within the dimensions of the seeing disk.

Fluctuations of the wave front coherence length, that is, the Fried parameter, r_0 , with time, limit the above restoration process at least in the current experimental set-up. Most of the data presented here were obtained in stable observing conditions, where this latter effect is small or negligible. Table 1 gives

the observing parameters for the two sources discussed below. Figs 2–4 present the quantity $|o_\lambda(f)|/|o_\lambda(0)|$ as determined by the reduction process for either source, versus spatial frequency in the direction ϕ_0 . Error bars represent one standard deviation determined from the signal-to-noise ratio on individual scans and the number of scans N_E .

Both sources present a low frequency peak which at $4.8 \mu\text{m}$ is more conspicuous on BN than on W3IRS5. The fraction of the total source power in this peak is small: biasing effects in the reduction process⁴ do produce such peaks but we have corrected the spectra for this effect². We will, however, only assign it tentatively to an extended component within the BN object and refrain from discussing at length its possible significance.

At the time of observation the $4.8 \mu\text{m}$ spectra were degraded by spurious 60 Hz system noise beyond $f \approx 2 \text{ arc s}^{-1}$. This was not the case for the $3.45 \mu\text{m}$ spectrum on Fig. 4, which shows a very good signal-to-noise ratio up to $0.95 f_c$. This particular spectrum illustrates well the capability of the speckle method to fully exploit the MTF of a large telescope. The oscillations appearing at high frequency in Fig. 4 are expected as the process (3) above divides noise by noise for $f \geq f_c$.

W3IRS5: this source is clearly resolved. We fit the spectrum with a function

$$\left| \frac{2J_1(\pi\alpha_0 f)}{\pi\alpha_0 f} \right|$$

corresponding to a source of uniform brightness with a diameter α_0 and find $\alpha_0 = 0.28 \pm 0.08 \text{ arc s}$. This large error bar is due to rather poor atmospheric conditions during this observation and is determined from the comparison of independent spectra. If the source had radial symmetry its radial profile $O(\alpha)$ would be completely restored.

If there is any flattening the configuration is less determined and would need scans at various angles ϕ . The above angular value α_0 refers only to the direction ϕ_0 .

A single size measurement for W3IRS5 is quoted by Werner *et al.*¹ to be 3,000 AU, or 1.08 arc s. As they gave no wavelength no comparison was possible.

Wynn-Williams *et al.*⁵ derive a 350 K black body energy curve between 2 and $5 \mu\text{m}$, which corresponds to a diameter of 0.2 arc s, in agreement with our measurement in the absence of flattening. Aitken and Jones⁶ have computed the extinction to the central object from the depth of the silicate feature and derive a corrected 600 K spectrum, to which corresponds a diameter of 0.24 arc s.

Bedijn *et al.*⁷ quoted an unpublished evidence for a double source in this object, for which we find no obvious evidence. Unfortunately, this result prevents them computing a model for W3IRS5. A less elaborate model is made by Finn and Simon⁸ which fits W3IRS5 spectrum with a central cold object having a black body temperature of 375 K. The corresponding $4.8 \mu\text{m}$ diameter is 0.33 arc s, which again agrees with the present measurement.

BN object: The $4.8 \mu\text{m}$ spectrum for the BN object is significantly different from the W3IRS5 spectrum. The central peak is slightly wider but again its power is a small fraction of the total. The main difference lies in the compact source which

Table 1 Observational parameters

Wavelength (M)	Object	Date (1978)	Magnitude m_λ	Flux (Jy)	T_0 (S)	Scan angle* (ϕ_0)	Reference star	$r_0^\dagger$ (cm)
$4.78 \mu\text{m}$	W3IRS5	Sept 11	0.8	80	552	150°	β Peg	110
$\Delta\lambda = 0.21 \mu\text{m}$	BN	Sept 11	-0.1	190	312	40°	μ Gem	240
(L)								
$3.45 \mu\text{m}$	BN	Sept 10	2.2	40	61	40°	μ Gem	165
$\Delta\lambda = 0.57 \mu\text{m}$								

Scan duration is $\tau = 38.4 \text{ ms}$.

* ϕ_0 is the angle between the scan direction and north, counted counterclockwise.

† r_0 is estimated using the low-frequency Korff asymptotic expression for the atmospheric MTF.

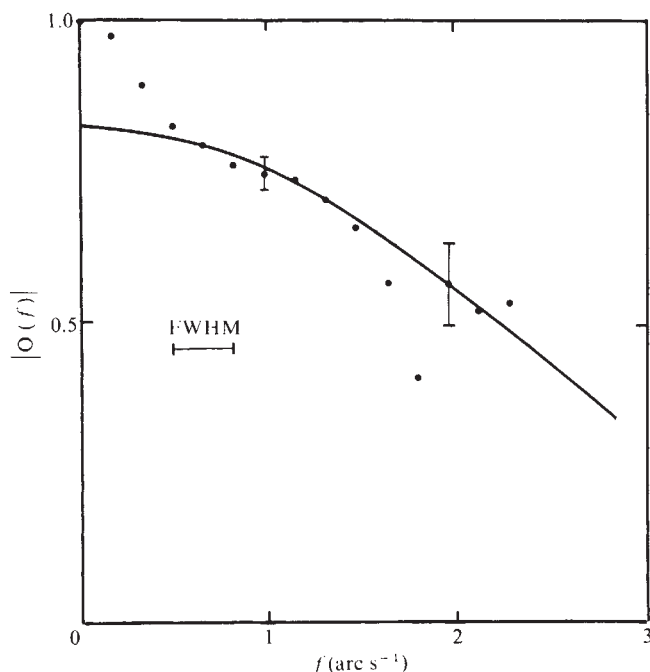


Fig. 2 W3IRS5 power spectrum $|O_\lambda(f)|/|O_\lambda(0)|$ versus spatial frequency in the direction $\phi_0 = 150^\circ$. Total observation time $T_0 = 552$ s. The full curve is the spectrum of a disk with uniform brightness and diameter $\alpha_0 = 0.28$ arc s. $\lambda = 4.8$ μm . FWHM is the frequency resolution.

seems barely resolved in this particular direction ϕ_0 . The diameter of a source of uniform brightness as above would be $\alpha_0(4.8 \mu\text{m}) \leq 0.1$ arc s if we assume the measured spectrum to depart from the spectrum of an unresolved source by less than 5% on the explored frequency range.

The central peak is fitted with a gaussian component corresponding to an extended gaussian emission with FWHM 1.5 arc s.

The 3.45 μm BN spectrum is easier to interpret. The central peak is present but is very dim and broader. It would again correspond to an extended emission with a gaussian intensity

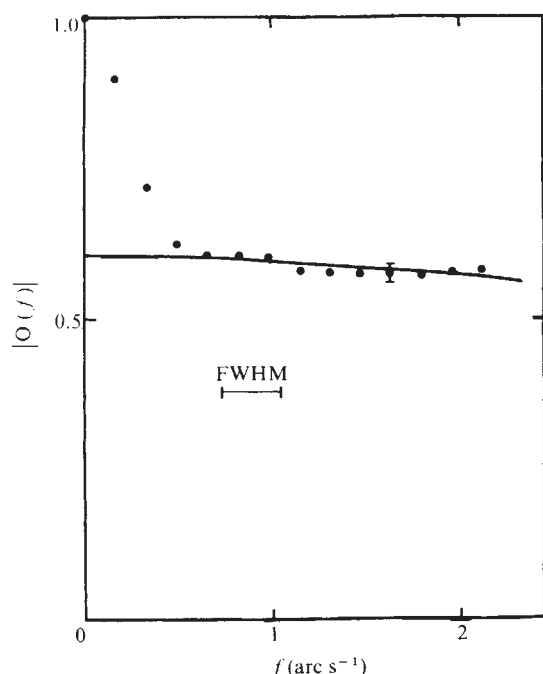


Fig. 3 BN power spectrum $|O_\lambda(f)|/|O_\lambda(0)|$ versus spatial frequency in the direction $\phi_0 = 40^\circ$. Total observation time $T_0 = 312$ s. The full curve is the spectrum of a disk with uniform brightness and diameter $\alpha_0 = 0.1$ arc s. $\lambda = 4.8$ μm .

profile and a FWHM of 0.9 arc s. The decrease of $|O_\lambda(f)|$ towards high frequencies is noticeable: Fig. 4 shows the spectrum of a disk having a uniform brightness and a diameter $\alpha_0(3.5 \mu\text{m}) = 0.1$ arc s. The telescope resolution in the Rayleigh criterion sense is given by $1.22 \lambda/D = 0.23$ arc s; the measured diameter is below this limit, because the high signal-to-noise ratio in the spectrum allows us to determine where it departs from being a point source spectrum.

Low⁹ has observed BN with spatial interferometry techniques and presents it as "unresolved" but does not specify the observation angle nor the wavelength nor the upper limit of the diameter. Becklin *et al.*¹⁰ fit the observed spectral distribution with a 530 K black body curve corresponding to a 0.1 arc s diameter at 4.8 μm . This agrees with our data if there is no flattening.

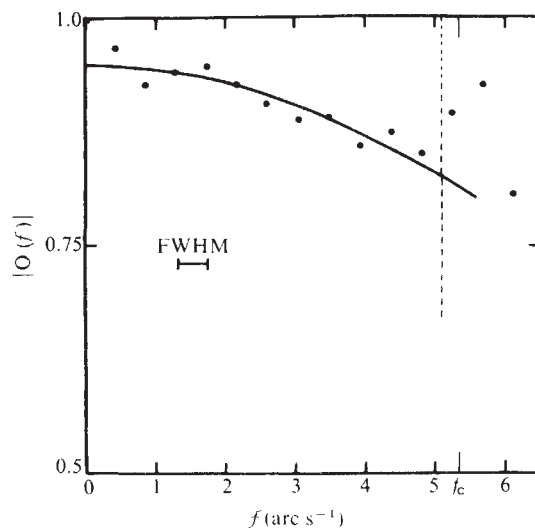


Fig. 4 BN power spectrum $|O_\lambda(f)|/|O_\lambda(0)|$ versus spatial frequency in the direction $\phi_0 = 40^\circ$. Total observation time $T_0 = 61$ s. The full curve is the spectrum of a disk with uniform brightness and diameter $\alpha_0 = 0.1$ arc s. The vertical dotted line at $f = 0.95 f_c$ sets the estimated confidence limit for the restoration process. $\lambda = 3.45$ μm .

A more elaborate treatment is given by Bedijn *et al.*⁷ who built a series of models for accreting stars. Applying these models to BN, these authors considered four possible cases, where a satisfactory fit of the observed spectrum was obtained, with a mass ratio of three between silicates and graphite grains.

The predicted 5- μm diameter—90% of the energy within it—varies from 0.42 to 0.12 arc s. The two first models are eliminated for dynamical reasons, our data only comply with the latter one. It corresponds to the lowest luminosity $2.9 \times 10^3 L_\odot$ for the embedded object, to a foreground extinction $A_V = 12.3$, and to a spectral type B3. The large model uncertainty on the luminosity—a factor six—is greatly reduced by measurement of the angular diameter.

As Bedijn *et al.*⁷ do not give the wavelength dependence of the size for $\lambda < 5 \mu\text{m}$, it is difficult to compare the 3.5 μm data. Nevertheless, one expects the diameter of the halo, if real, to slightly decrease, as observed, owing to the contribution of inner

Table 2 Measured angular diameter (in direction ϕ_0)

	Wavelength (μm)	ϕ_0	Extended* halo envelope (FWHM, arc s)	Central source diameter (uniform disk) arc s
W3IRS-5	4.8	150°	1.5	0.28 ± 0.08
			Very weak	
	4.8	40°	1.5	≤ 0.10
BN	3.45	40°	0.9	0.10
			Very weak	

*Interpret with caution.

layers at a higher temperature and Yorke (personal communication) predicts the existence of a halo surrounding the central object for $\lambda > 2.5 \mu\text{m}$. More elaborate models are now needed, especially to compare the wavelength—and radial—dependence of the emerging intensity in the region of possible accretion.

These measurements confirm that two likely candidates for pre-main-sequence objects in the solar neighbourhood are high luminosity sources embedded in a very compact heated shell. Following Bedijn *et al.*⁷ it is unlikely that such a phase can be sustained for longer than 10^5 yr. Other evidence for a transient expansion phenomenon around the BN object was recently pointed out by Hall *et al.*¹¹ in the discovery of an expanding shell observed in the lines of the CO molecule.

Although these first measurements are restricted to a single radial direction, and do not yet answer the crucial question of the flattening in the accretion disk and its possible rotation, the method proves that sizes of the order of 40 AU are within reach in the thermal IR: radiative transfer problems and inner dynamics of accretion cores can therefore be studied observationally.

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Quasi-biennial variation of the solar neutrino flux and solar activity

THE observed flux of the solar neutrinos is lower by a factor of five than that theoretically estimated from the study of the standard model of the solar interior¹. To explain this discrepancy various theories have been proposed which disregard some of the assumptions inherent in this model. However, no theory has been able to reduce the theoretical fluxes to the observed ones^{2,3}. The experimental data obtained by Davis *et al.*¹ indicate that this discrepancy is real, so that these data should be seriously considered in any future research on the solar neutrinos. They have published these data for each period that they have observed since 1968, (Fig. 1)^{4,5}. We have used their data on the solar neutrino fluxes since 1968 to study whether the neutrino flux has any tendency to vary periodically during the solar cycle of 11 yr. The dependence of the neutrino flux on the solar activity, namely, the phase of the solar cycle, was suggested by Sheldon⁶. He explained that, in the late 1960s, the neutrino production rate in the solar core seemed to be dependent on the degree of the solar activity, as he could only refer to the early results on the neutrino flux observations. It is

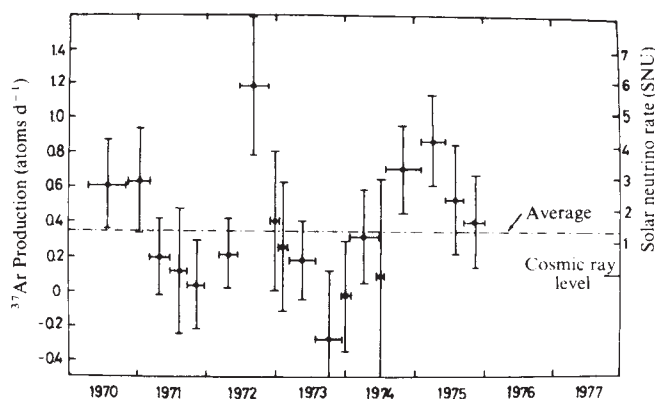


Fig. 1 The observed fluxes of the solar neutrinos for the years 1970–76 (Davis *et al.* 1976).

shown here that this idea is not supported by the data on the neutrino flux obtained since 1968.

The neutrino flux may have been varying quasi-biennially throughout the solar cycle since 1968 (ref. 7), and this flux variation seems to be independent of the mean trend of the variation of the solar activity for 11 yr. Therefore, this 'quasi-biennial' variation of the neutrino flux must be related to some feature of the solar phenomena, because such a variation with a period as short as 2 yr, may produce some phenomena on the solar photosphere which are causally connected with those taking place in the interior of the Sun. One of these must be the variation of the relative sunspot numbers because they are

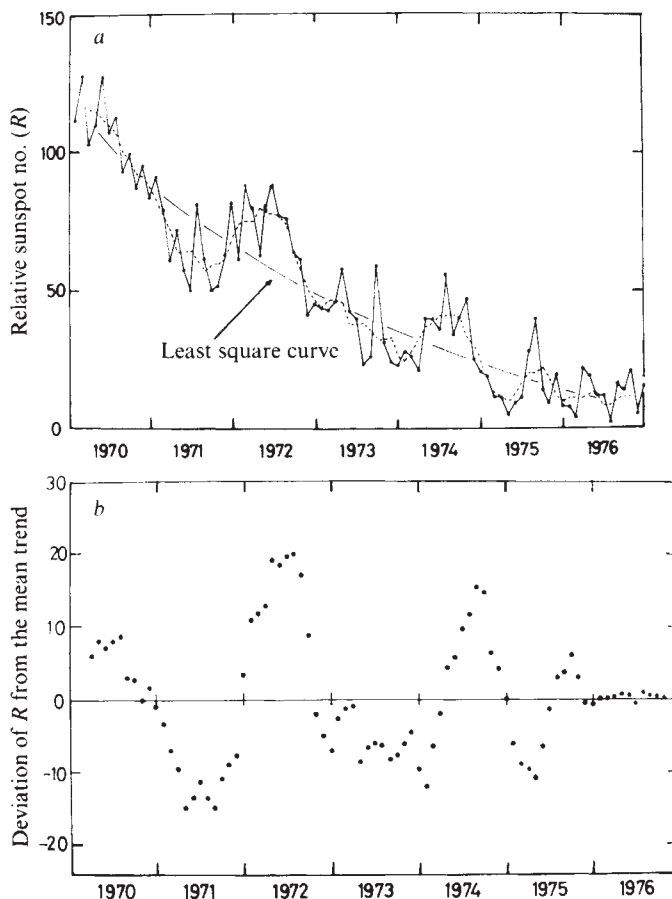


Fig. 2 a, The monthly mean values of the relative sunspot numbers for the years 1970–76. The moving average for 5 months is indicated by the dashed line. The mean trend of the variation of the sunspot numbers is shown by the solid line; b, The deviation of the relative sunspot numbers from the mean trend shown in (a).

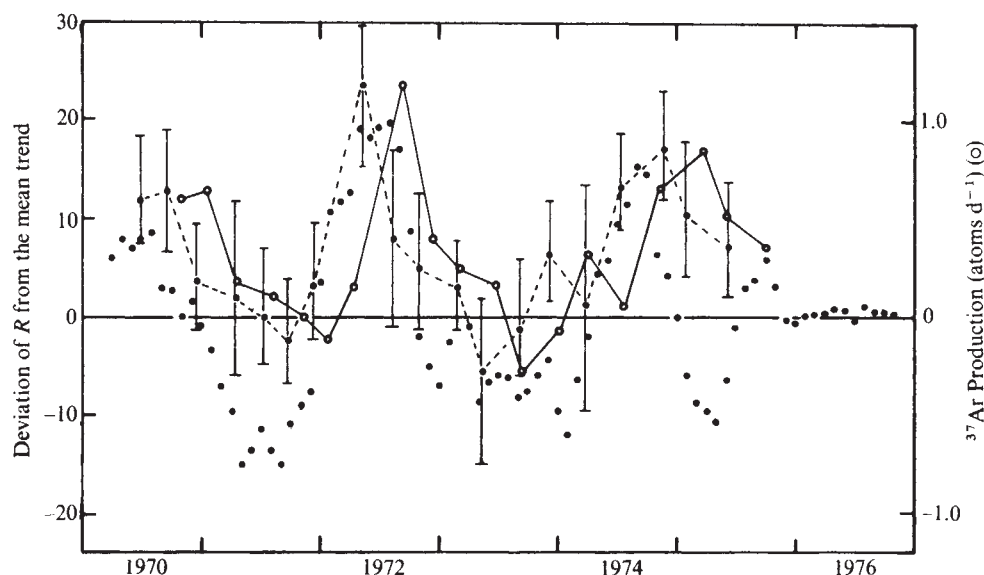


Fig. 3 The relationship between the variation of the solar neutrino flux and the deviation of the solar activity from the mean trend for the years 1970–76. The open circles indicate the mean observed fluxes of the solar neutrinos shown in Fig. 1. The dashed lines give those fluxes shifted earlier by 4 months.

produced by some instability associated with hydromagnetic convective motion beneath the photosphere.

According to the power spectral analyses by Shapiro *et al.*⁸, the relative sunspot numbers vary with a period of 25.7 months. Compared with the result shown in Fig. 1, it turns out that this period is very similar to that for the quasi-biennial variation of the solar neutrino flux.

To determine whether a quasi-biennial variation for the relative sunspot numbers exists between 1970 and 1976, the moving average for 5 months was first obtained for the monthly mean relative sunspot numbers as shown in Fig. 2a. This shows that the relative sunspot numbers deduced from this average have a clear tendency to vary quasi-biennially while superimposing the mean trend, shown by the thick line, which represents the pattern for the 11-yr variation of these sunspot numbers. From the result shown in Fig. 2a, the deviation of these numbers from this mean trend can be evaluated by subtracting the numbers for the mean trend from the observed sunspot numbers averaged over 5 months with respect to each month.

The relative sunspot numbers deviation from the mean trend is shown in Fig. 2b. This shows that these numbers vary quasi-biennially, although the data for 1976 do not show such tendency clearly because this year was at the solar activity minimum. The time-series analytical method has then been applied to estimate the length of an apparent period for this quasi-biennial variation as shown in Fig. 2b. The result clearly indicates that the relative sunspot numbers for these years also vary with a period of 25.3 months, which is almost the same as obtained for other years⁸. This suggests that the solar activity always varies with a period of about 25 months, which is independent of the phase of the solar activity cycle. Furthermore, the geomagnetic disturbances are controlled by the quasi-biennial variation of the solar activity⁹.

Comparing the result of Fig. 2b with the observed data on the neutrino flux shown in Fig. 1, it follows that when the deviation is positive the observed flux of the solar neutrinos seems to be generally very high and vice versa, if we neglect small time difference.

To examine this relationship quantitatively, the observed fluxes of the solar neutrinos have been superimposed on the result shown in Fig. 2b. The result is shown in Fig. 3, where the open circles are the observed values of the solar neutrino fluxes, which are connected with the solid lines, although the error bars have been omitted. The filled circles connected with the chain lines indicate the observed fluxes shifted earlier by 4 months, for which the error bars are added to show a correlation between these fluxes and the deviation of the relative sunspot numbers. Figure 3 shows that although the statistical variation of the solar

neutrino fluxes is quite large, these fluxes, except for those of 1976, vary in phase with the variation of the sunspot numbers deviated from their mean trend. This result is, however, unexpected when we use the standard model of the Sun to investigate the production process of the solar neutrinos. This result suggests that this process is causally related to some unknown process taking place in the interior of the Sun, which must also be responsible for the quasi-biennial variation of the solar activity. As the cause of the solar activity is associated with large-scale internal motion associated with some unstable convective modes, this process may be accompanied by some form of the motion. Furthermore, the result shown here suggests that this motion can penetrate into the region where the thermonuclear fusion is taking place and thus disturb the stable burning processes of its hydrogen and the helium-3. No theory on such a motion has yet been proposed but our result means that further investigation is needed on the large-scale internal motion of the Sun, which causally connects the fusion processes with the quasi-biennial variation of the solar activity.

The standard model does not provide any means to investigate such convective motion penetrable to the nuclear-burning central core of the Sun. However, some oscillatory modes of motions may exist with longer periods not previously observed. One of these may vary with a period of about 2 yr if the quasi-biennial variation of the relative sunspot numbers has to be produced from some modes of the convective motion beneath the solar photosphere. Thus the continued observation of the solar neutrino flux for several solar cycles would give us an important clue when examining the accuracy of the standard model and its limitations in investigating the interior of the Sun in relation to the nuclear-burning processes.

There is evidence, in fact, that the azimuthal component of the convective motion, measured as the angular velocity, seems to be varying quasi-biennially during the last solar cycle no. 20 (1965–76) (ref. 7). This motion, therefore, seems to be associated with the convective motion mentioned above. However, such motion can never be derived from the study of the standard model of the Sun.

These results indicate that the production rate of the neutrinos in the solar interior varies quasi-biennially and may be causally associated with the convective motions responsible for the quasi-biennial variation of the relative sunspot numbers.

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Tensile strength of water

THE measured tensile strength of water has long been known to be significantly less than theoretical predictions and the reduced strength is normally attributed to the presence of solid impurities that serve as nucleation sites for rupture of the liquid¹. A favourite method of measuring tensile strength is through the dynamic stressing of a liquid by an acoustic field. At sufficiently large values of the peak negative acoustic-pressure amplitude the liquid ruptures and forms a rapidly growing vapour cavity that collapses violently during the positive portion of the cycle. This cavity collapse is normally violent enough to be observed easily with the unaided eye or ear. Such an event is termed the acoustic cavitation threshold and is a measure of the tensile strength of a liquid. Although some successes have been obtained in achieving measured values of the tensile strength comparable to theoretical predictions for extremely small samples² or after extensive filtration³, I know of no current theory for the prediction of values of the dynamic tensile strength for ordinary distilled water. Using a modified version of a recent theory by Apfel⁴, an equation is presented here that correctly predicts the variation of the acoustic cavitation threshold of water with surface tension, dissolved gas content and temperature.

It is assumed that there exists in the body of the liquid numerous nonpolar solid impurities that contain cracks and crevices in which gas pockets may be stabilised against dissolution. Scanning electron micrographs have been made of filtered solid impurities and show innumerable sites for possible gas-pocket stabilisation⁵. Figure 1a is a typical micrograph of a filtered particle. Following a theory of Apfel⁴, and others^{1,6}, it is possible to determine requirements on the threshold acoustic pressure required to nucleate a free gas bubble that will serve as a preferential site for liquid rupture. Apfel⁴ expresses the threshold pressure P_A , as

$$P_A \geq P_H - \gamma P_G - P_V + (P_H - P_G - P_V) \left| \frac{\cos(\alpha_R - \beta)}{\cos(\alpha_A - \beta)} \right| \quad (1)$$

for conditions such that $\alpha_R > \beta$. In equation (1), P_H is the hydrostatic pressure, P_G the equilibrium pressure of the gas dissolved in the liquid, P_V the vapour pressure, γ a constant with value between 0 and 1 which measures the ability of the nucleating gas pocket to maintain the equilibrium gas pressure, β the half-angle of the conical crevice, and α_A and α_R are the advancing and receding contact angles of the liquid–gas interface in the crevice. This equation has been modified as follows to apply it to the experimental situation. It is assumed that during the stabilisation process the interface advances to a specified position and stops. This position determines the diameter of the crevice at the position where the interface meets the crevice wall. It is further assumed that the crevices stabilise in water at normal surface tension and later reduction of surface tension by addition of surfactant does not affect the position of the stabilised interface. The shape of the interface may change but its position in the crevice will not. This assumption allows us to express the quantity $|\cos(\alpha_A - \beta)|$ as a constant, dependent only on the initial value of the surface tension. A second modification of the equation involves the nucleation process. The receding contact angle is affected by addition of a surfactant and its dependence

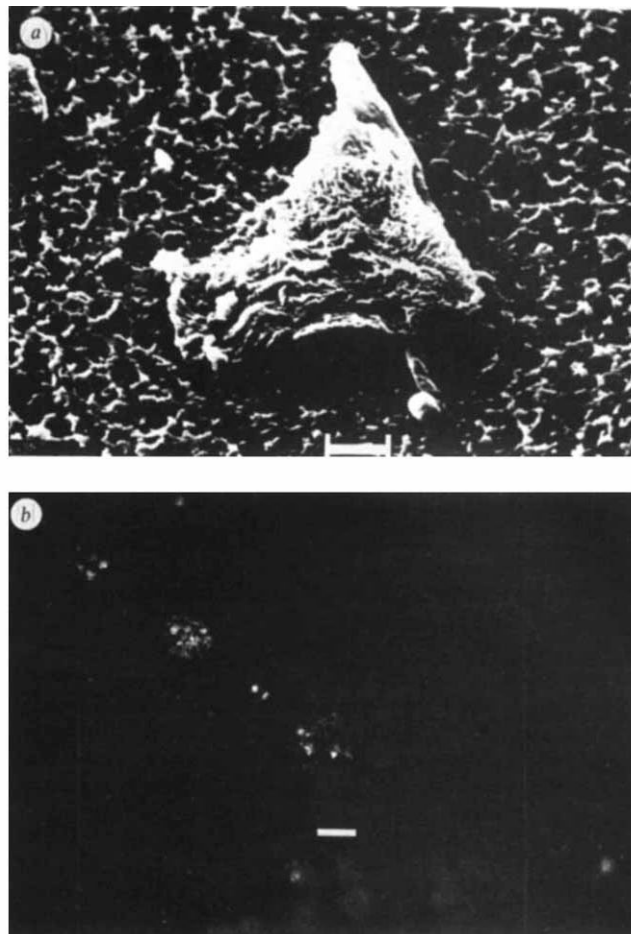


Fig. 1 a, Scanning electron photomicrograph of a solid impurity. The particle was removed by filtration of distilled water with a 0.45- μm pore size filter, shown as the mottled area in the background. Note the ragged appearance of the surface showing the numerous possible nucleation sites. Scale bar, 5 μm . b, Photograph showing creation and resultant motion of a transient cavity. The cavity was initiated at the centre of a resonating piezoelectric cylinder and was propelled outwards by radiation-pressure forces. Here the flash interval was 2.4 ms, the acoustic pressure amplitude at the antinode was 4.7 bar, and the maximum velocity achieved was about 100 cm s^{-1} . Scale bar, 1 mm.

on surface tension must be specified. This is done by writing $\cos(\alpha_R - \beta) = \cos[\alpha_E - (\alpha_H + \beta)]$ where α_E is the equilibrium contact angle and α_H is the hysteresis between the equilibrium and the receding contact angle. Such a modification allows us to express the receding contact angle in terms of the surface tension. Bargeman and Van Voorst Vader⁷ have found that α_E can be expressed as

$$\cos \alpha_E = -1 + C/\sigma \quad (2)$$

where σ is the surface tension and C is a constant that depends on the surface properties of the solid. For nonpolar solids such as paraffin, and beeswax, $C \approx 50$.

The acoustic cavitation threshold can thus be expressed in terms of measurable parameters as

$$P_A = (P_H - P_V - \gamma P_G) + \frac{(P_H - P_V - P_G)}{\delta} \times \{\cos \phi (C/\sigma - 1) + \sin \phi [1 - (C/\sigma - 1)^2]^{1/2}\} \quad (3)$$

To apply this theory to ordinary water, the unknown parameters, γ , δ and ϕ must be specified. γ is 1.0 for situations in which the gas pressure in the nucleating cavity is maintained at

equilibrium with that in the fluid. As a precise measurement of this constant is impossible, it is assumed that equilibrium will be maintained and thus $\gamma = 1.0$. δ is a parameter which measures the initial value of the advancing contact angle, and is given by $\delta = |\cos(\alpha_A - \beta)|$. Values for the advancing contact angle of

water on nonpolar solids are of the order of 106° . Bargeman⁸ lists values of α_A for four nonpolar solids with an average of 106° , standard deviation 6° ; water on paraffin wax is known to be approximately 106° (ref. 9). α_A was thus selected to be 106° . The crevice angle β is assumed to be small; crevices need small angles for stabilisation; a value of $\beta = 14^\circ$ was selected to give $\delta = 0.035$. Values of the hysteresis angle of water plus surfactant on nonpolar solids were measured by Furmidge¹⁰. He obtained values ranging from 14° to 44° for water plus surfactants on white beeswax. To obtain a good fit to the data, α_H was chosen to be 18° ; hence $\phi = \alpha_H + \beta = 32^\circ$.

The incipient cavitation threshold was measured for acoustic cavitation in distilled water. A hollow piezoelectric cylindrical transducer was used that was open at one end and closed at the other with a thin glass window (thickness 0.01 cm). The thin glass window approximated a pressure-release boundary and the cylinder was driven in a $(r, \theta, z) = (3, 0, 3)$ resonant mode that generated cavitation only along the axis of the cylinder and only at the middle antinode. The height of the cylinder was ~ 7.5 cm, the inside diameter was 7.0 cm, the thickness was 0.5 cm. Figure 1b shows a photograph of a cavitation event that was obtained under stroboscopic illumination. The cavity was created along the axis of the cylinder, approximate centre of photograph, and was propelled outwards by radiation pressure forces¹².

The variation of the cavitation thresholds is shown in Fig. 2a-c respectively: note the excellent agreement with theory. The selection of the parameters γ , δ and ϕ was varied slightly to obtain a best fit but was restricted to lie within the range of measured values. The theory shows no variation with frequency; measurements were made in the low kilohertz region where the measured threshold does not depend upon frequency¹³. For higher frequencies, cavity dynamics are important in determining the threshold^{4,14}.

It is seen that the dynamic tensile strength of distilled water can be accurately predicted if proper account is made of the solid impurity content of the liquid.

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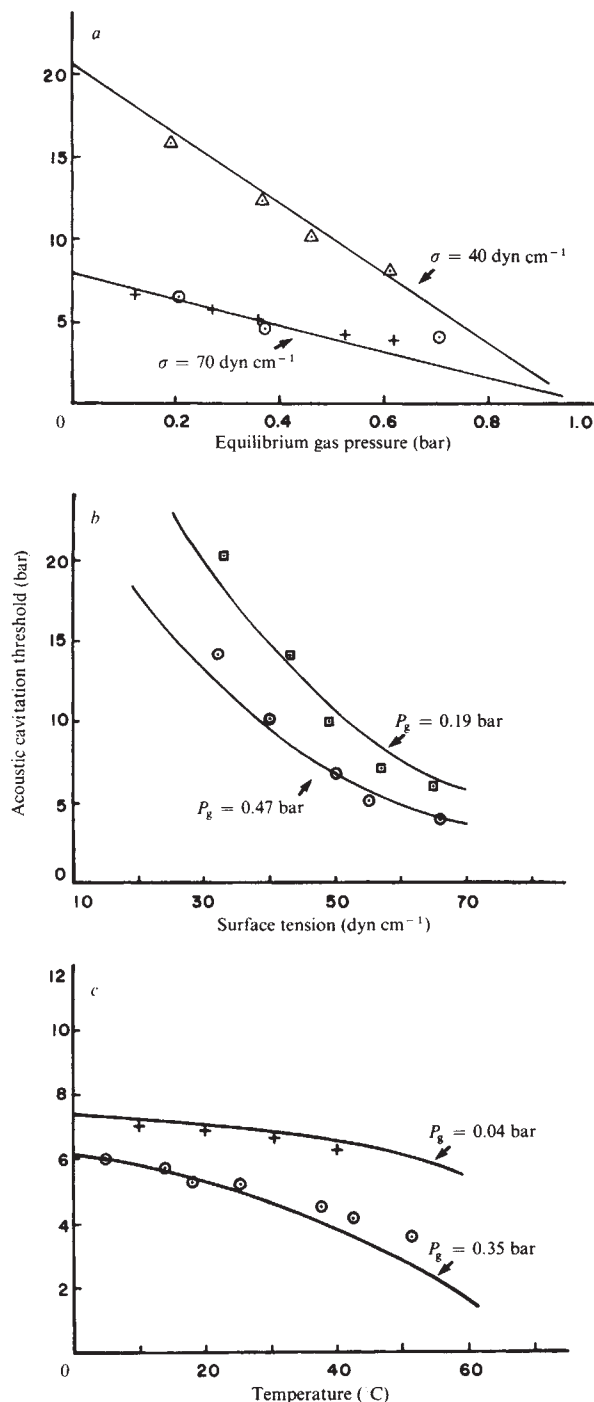


Fig. 2 Variation of the acoustic cavitation threshold of water with (a) dissolved gas content for two values of the liquid surface tension; (b) with surface tension for two values of the equilibrium gas pressure; and (c) with temperature for two values of the equilibrium gas pressure. The curves are for equation (3) with $\gamma = 1.0$, $\delta = 0.035$ and $\phi = 32^\circ$. In (a) Δ and $+$ are experimental measurements from this study and $\circ$ points are values obtained by Strasberg⁶; the frequency in (a) and (b) was 36 kHz and the temperature 25°C . In (c) the frequency was 22 kHz and the surface tension was 70 dyn cm^{-1} ; $+$ points are the normalised values of Galloway¹¹; $\circ$ are measurements from this study. The temperature dependence of the vapour pressure P_v and the equilibrium gas pressure P_g have been inserted into equation (3).

Simulated lidar return from a one-dimensional stratospheric aerosol model

RESULTS of theoretical calculations on lidar backscatter from the stratospheric aerosol are presented here. The calculations use the size distribution, particle number density and particle composition predicted by a one-dimensional model of the stratospheric aerosol layer. The theoretically calculated backscatter profile is compared with experimental results obtained from actual lidar observations of the layer.

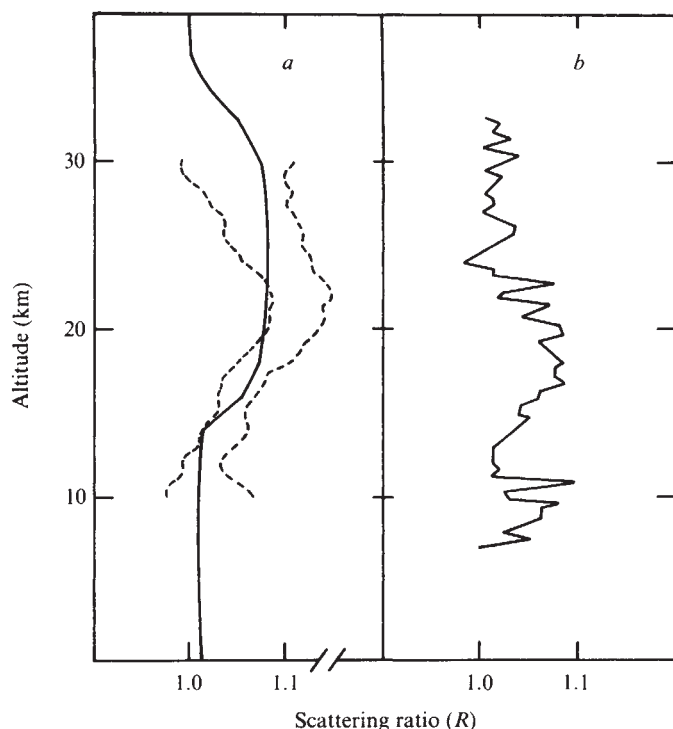


Fig. 1 *a*, Scattering ratio (R) as a function of altitude calculated from aerosol model. Dotted lines represent the range of experimental uncertainty (from ref. 11). *b*, Lidar backscattering ratio obtained 20 September, 1978 with Langley Research Center 48" lidar system.

The one-dimensional stratospheric aerosol model used here is described fully elsewhere¹; therefore we shall give only a few pertinent details. The model assumes that the primary source of sulphur to the stratosphere is biogenic OCS released at ground level². This OCS diffuses into the stratosphere where it is photodissociated, leading to the production of gas phase sulphuric acid. Aerosol droplets are formed by heteromolecular-heterogeneous nucleation of gaseous sulphuric acid and water onto pre-existing solid particles of tropospheric origin. The aerosol particles are spherical, liquid $\text{H}_2\text{SO}_4\text{-H}_2\text{O}$ solution droplets with solid cores. The particles undergo the physical processes of condensation, evaporation, coagulation, sedimentation and vertical eddy mixing³. The model uses these chemical and physical mechanisms to predict altitude profiles for all important aerosol parameters, including the size distribution, number concentration and amount of sulphuric acid in the drops.

Model predictions have been compared with *in situ* measurements of the stratospheric aerosol¹, and agreement is good. Consequently, the model should predict aerosol backscatter ratios reasonably well. However, *in situ* measurements are quite rare above 25 km so they cannot be used to check model results at high altitudes. Therefore, we wish to show that reasonable lidar backscatter profiles can be predicted theoretically using the aerosol model. We also use model results to perform a few simple theoretical 'experiments' to determine how lidar observations will be affected by changes in certain properties of the aerosol layer.

The model simulates a 'background' aerosol; that is, sudden injections of sulphuric gases and particles were not incorporated in the model. Thus, the model results represent the stratospheric aerosol during volcanically quiescent periods. Fortunately, there is a fairly comprehensive lidar record of the aerosol layer over the past decade^{4,5} and much of the data have been obtained during periods of little or no volcanic activity. The last volcano which injected a significant amount of material into the stratosphere was Volcán de Fuego which erupted in October 1974. As

shown by McCormick *et al.*⁶ the volcanic debris in the stratosphere which could be attributed to Fuego decayed away with a $1/e$ time constant of about one year. Before Fuego, a large amount of volcanic matter was injected into the stratosphere by Agung (March 1963). However, just before Fuego, and at present (November 1978), lidar backscatter studies show an aerosol layer close to 'background' levels. Therefore, our theoretical results are compared with data taken either shortly before Fuego or near the present date.

The aerosol parameters used in our calculations are the following: the lidar backscatter intensity was calculated using a Mie model to evaluate the aerosol backscatter coefficient (f_a) for wavelengths $\lambda = 0.6943 \mu\text{m}$ (lidar wavelength) and $\lambda = 1.06 \mu\text{m}$. Both the Mie model and the aerosol model use standard atmosphere⁷ mid-latitude profiles for temperature, density and water vapour. Using the index of refraction data given by Palmer and Williamson⁸ and model results, we determined the altitude dependent index of refraction of the sulphuric acid aerosols. The values varied from 1.41 at 10 km to 1.44 at 38 km. We assumed that the index of refraction of the particles was that of the sulphuric acid-water shell, an assumption which is valid for particles with small cores⁹. The model distinguishes between sulphuric acid aerosol droplets and solid 'condensation nuclei' of tropospheric origin. For the condensation nuclei the real part of the index of refraction was taken to be 1.50, independent of altitude¹⁰.

The results of our calculations are shown in Figs 1-4. These are profiles of the lidar scattering ratio, R , defined by

$$R = \frac{f_a + f_m}{f_m}$$

where f_m is the molecular (Rayleigh) backscattering coefficient.

Figure 1 shows the calculated lidar backscatter ratio at $\lambda = 0.6943 \mu\text{m}$ from aerosol droplets and condensation nuclei between 10 and 40 km altitude. On the same plot we give the range of $\pm$ one standard deviation about the mean measured in 16 lidar observations taken in the period June 1973 to March 1974 by Russell *et al.*¹¹. This was shortly before Fuego so presumably the stratospheric aerosol was primarily non-volcanic in origin. As Fig. 1 shows, the model prediction is in good agreement with experimental results. It should be noted, however, that individual lidar returns show a great deal of structure which is not present in the model. To illustrate this, a typical lidar backscatter ratio profile is shown in Fig. 1*b*. The structure is probably a result of small scale temperature and

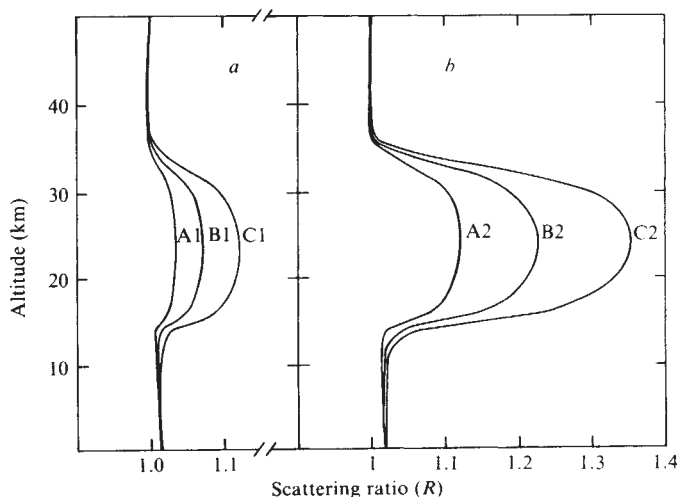


Fig. 2 *a*, Calculated scattering ratio profiles for different values of real part of index of refraction (η) for wavelength $\lambda = 0.6943 \mu\text{m}$. Curve A1, B1, C1 are for $\eta = 1.3, 1.4, 1.5$, respectively. *b*, Scattering ratio profiles for wavelength $\lambda = 1.06 \mu\text{m}$. Curves A2, B2, C2 are for $\eta = 1.3, 1.4, 1.5$, respectively.

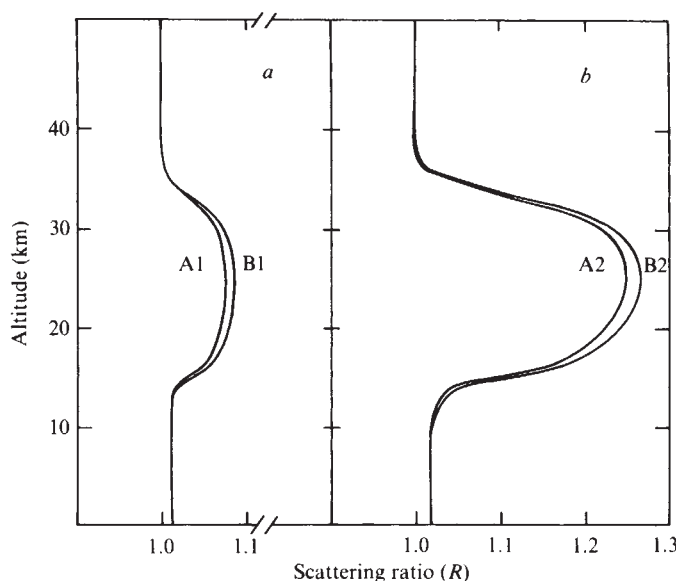


Fig. 3 *a*, Calculated scattering ratio profiles for different values of imaginary index of refraction (k) for $\lambda = 0.6943 \mu\text{m}$. Curves A1 and B1 are for $k = 0.00$ and 0.1 , respectively. *b*, Scattering ratio profiles for $\lambda = 1.06 \mu\text{m}$. A2 and B2 are for $k = 0.00$ and 0.1 , respectively.

water vapour fluctuation and the advection of particles due to stratospheric dynamics. These phenomena are not included in the model. Consequently we can expect agreement between the model and the average of a number of observations, but not for a particular lidar return.

The agreement between model and observation shown in Fig. 1 is particularly striking when one considers the effect of volcanic activity on the lidar returns. For example, post Fuego observations^{5,6} gave scattering ratio (R) profiles that were very sharply peaked and reached maximum values >4 . The pre-Fuego data shown in Fig. 1 (and present day observations of the layer) give maximum values of R of about 1.08.

The calculated lidar signal in Fig. 1 indicates the top of the layer around 35 km. The microphysical processes incorporated in the model result in the evaporation of the sulphuric acid droplets at about 34 km. However, there are some droplets above that altitude due to mixing. Also, the evaporation of the droplets is a source of condensation nuclei (cores). Therefore, the predicted lidar return does not drop off until somewhat above the evaporation level. Lazrus and Gandrud¹² have reported the existence of sulphates up to 36 km, which is slightly higher than indicated by model results or by measurements made with lidar and other methods.

To determine the sensitivity of lidar observations to the index of refraction (η) we carried out calculations varying η for both the aerosol particles and the condensation nuclei. Changes in η for the condensation nuclei did not affect the results because they are so small that they do not contribute significantly to the backscatter. Even the presence of highly absorbing condensation nuclei will hardly affect the lidar return. To verify this we varied the imaginary part of the index of refraction (k) of the condensation nuclei from 0.0 to 1.2 and obtained backscatter profiles which were nearly identical.

However, changes in η for the aerosol particles do affect the backscatter ratio profile. Figure 2*a* shows calculated profiles assuming η to be 1.3, 1.4 and 1.5. The curves obtained for $\eta = 1.3$ and 1.5 fall outside the range of values given in Fig. 1. This result is significant as it indicates that the index of refraction of the stratospheric particles could be evaluated from lidar returns. (Such an analysis should only be undertaken if the results of many lidar returns are averaged and the atmospheric conditions are comparable to those used in the model. Also one

should have independent information on the size distribution and number density of the particles as these also affect the return). Note that η for ammonium sulphate is slightly greater than 1.5 at $0.6943 \mu\text{m}$. Thus, the results given in Fig. 2*a* suggest that the aerosol particles are unlikely to be composed of ammonium sulphate, as was believed by earlier investigators of the stratospheric layer.

The value of k for sulphuric acid-water solutions at $0.6943 \mu\text{m}$ is of the order of 10^{-8} and in most of our calculations we used a zero imaginary index. However, to investigate the effect of a large k on lidar backscatter we performed some calculations with $k = 0.1$. It must be emphasised that such a value is many orders of magnitude larger than expected. Nevertheless, as shown in Fig. 3*a*, the effect on the backscatter ratio profile is not large.

It has been suggested that multi-wavelength lidar studies of the aerosol layer should be carried out. Figures 2*b* and 3*b* show the effect of observing the stratospheric aerosol with a $1.06 \mu\text{m}$ wavelength lidar. The most salient characteristic of these figures is the fact that the scattering ratio at $1.06 \mu\text{m}$ is much larger than at $0.6943 \mu\text{m}$. But a comparison of Fig. 2*a* and *b* shows that the relative change in scattering ratio with changes in η is nearly the same for both wavelengths. Thus lidar observations at these two wavelengths would apparently give little additional information on the index of refraction. Similarly, comparing Fig. 3*a* and *b* we note that a change in λ gives a larger absolute difference between the absorbing and non-absorbing cases, but the relative difference between the two cases is only slightly greater for the larger wavelength.

Meteoritic ablation in the mesosphere might be a significant source of small particles in the stratosphere. To determine the effect of increasing the number of small particles on the lidar return, we incremented the number of condensation nuclei of size $0.01 \mu\text{m}$ radius to a mixing ratio of 800 particles mg^{-1} air above 30 km, and to follow the profile given by Käsela *et al.*¹³ below 30 km. This enormous increase in small particles had no discernible affect on plotted backscatter ratio profiles. The reason is, of course, that the $0.6943 \mu\text{m}$ backscatter is quite insensitive to small particles. Consequently, one cannot expect lidar studies to determine whether meteoritic ablation contributes significantly to the particulate loading in the stratosphere.

Finally, to evaluate changes in temperature on the lidar return, we calculated the backscatter profile using aerosol model results

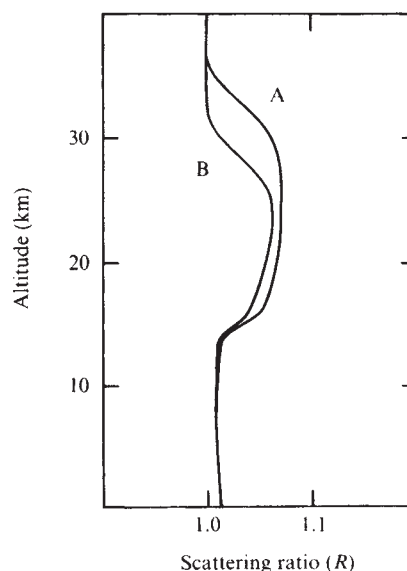


Fig. 4 Calculated scattering ratio for different stratospheric temperature profiles. Curve A is for spring/autumn 45° latitude standard atmosphere. Curve B is for July 75° north latitude standard atmosphere.

obtained with a 'warm stratosphere' temperature profile (USA Standard Atmosphere, July, 75° N latitude). The results, presented in Fig. 4, indicate that the lidar returns drop off at lower altitudes, and there is a small decrease in maximum values of the scattering ratio. Calculations using a 'cold stratosphere' (high latitude winter) show a profile extending to higher altitudes, as expected. The model assumes a smoothly varying temperature profile and does not include advection so the sharp peaks in scattering ratio at low temperatures which were noted by McCormick, *et al.*⁶ do not show up in our calculations.

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On the detection of lunar volatile emissions

ONE of the most interesting scientific results of the Apollo programme was the discovery that the Moon sporadically releases radiogenic gases¹⁻⁴. It is likely (although not yet proved) that these emissions are correlated with other detectable lunar activity such as shallow moonquakes^{5,8} and the elusive visible events known as 'lunar transient phenomena' or LTPs^{6,7}. Runcorn has argued⁸ that these three types of lunar activity are linked through tidal triggering of cylindrical fault systems around the circular mare, which results in gradual settling of the mascons and venting from the cracks. Considerable evidence exists which favours such a model, including seismic⁹, photogeologic¹⁰ and electromagnetic induction¹¹ data. However, a search for sporadic venting using the Apollo lunar surface Suprathermal Ion Detector Experiments (SIDEs) was unsuccessful¹². This note points out that the SIDE instruments lack the sensitivity to detect even large gas emissions if the venting occurs primarily at a few well-defined LTP sites, and suggests specific flight instruments for the proposed ESA Polar Orbiting Lunar Observatory (POLO) mission^{13,14} which could detect active venting, would help to determine the energy source for LTPs, and would increase our knowledge of lunar geophysics.

It is known that ⁴⁰Ar, ⁴He, and ²²²Rn are outgassed from the lunar interior^{2,4}, where they are formed from the decay of ²³⁸U, ⁴⁰K and ²³²Th. Based on the Rn data, Hodges argues² that the characteristic time for the radiogenic gas release process is ~70 d, with the Rn carried to the lunar surface by the more plentiful Ar and He in an abundance ratio of approximately 137,500:37,500:1 (He:Ar:Rn). The total loss rate for these species from the Moon is about 1.6×10^{30} atoms yr⁻¹ or 1.4×10^4 kg yr⁻¹ with a mean atomic weight of 11.6 AMU. Vondrak³⁰ has calculated the maximum column density ρ of vented gas at an arbitrary distance, R , from a source on the lunar surface. For both direct flux and diffusive transport models, he finds $\rho = S/\epsilon\pi R^2$ provided the gases are not strongly absorbed by the surface, where S is the number of particles released from the (assumed) impulsive point source. Vondrak argues that a gas release would be readily detectable if it increased the column density at one of the three SIDE sites by about a factor of five above the normal lunar dayside density of 10^{12} atoms cm⁻². If it is assumed that the vented gases are primarily the He and Ar which are known to be released, rather than CO, CO₂, H₂O and so on as in Vondrak's study (mean atomic weight 32 AMU), his results are modified slightly. Table 1 shows the minimum venting required from one of the six most prominent LTP sites^{6,7} to be detected at a SIDE location. These results indicate that if the lunar He, Ar and Rn vented from the interior are released sporadically at one or more of these LTP sites, it is unlikely that the gas release would have been detected by a SIDE instrument. For example, if half of the released lunar radiogenic He and Ar were to be vented sporadically from the crater Aristarchus and its neighbourhood (where more than one-third of all reported LTPs have been detected)⁷, these events would go unnoticed by the SIDEs. Of course, it is expected that the gases produced in the interior are vented from the Moon at several locations, so that a very large fractional release of the yearly He-Ar-Rn loss is required at one site close to a SIDE to be detectable (for example, one-sixth of the yearly loss vented at the crater Alphonsus in one brief event would be observable at the Apollo 14 site). Therefore the SIDE data are compatible with the other Apollo measurements which indicate sporadic He, Ar and Rn releases.

If the LTP are related to sporadic venting of these radiogenic gases, the detection of such events should be one of the mission objectives of the proposed POLO spacecraft. Previous studies have demonstrated that great difficulties arise in attempts to find energy sources at the lunar surface which could adequately explain the LTP^{7,15}, which in some cases must exhibit a specific lunar surface brightness of at least $1-10$ W m⁻² (optical) to be observable from the Earth¹⁶. One process¹⁶ which may be capable of exciting local clouds of He and Ar to the required brightness levels is the Alfvén critical velocity effect^{17,18}. This effect is a nonthermal ionisation process which occurs when a plasma moves through a neutral gas in the presence of a magnetic field at a velocity greater than $\sim (2e\psi/m)^{1/2}$ where ψ and m are the ionisation potential and mass of the neutral atom respectively. The exact mechanisms which transform the relative ion-neutral kinetic energy into heating of the free electrons which must do the ionisation are not fully understood^{19,21}, but the existence of critical velocity phenomena has been

Table 1 Minimum He-Ar release from LTP sites which would be detectable at SIDE locations, after Vondrak³⁰

Vent location	Gas release (kg)		
	Apollo 12	Apollo 14	Apollo 15
Aristarchus	1×10^4	1.3×10^4	1.8×10^4
Alphonsus	4×10^3	2.4×10^3	1.4×10^4
Plato	2.6×10^4	2.5×10^4	5.8×10^3
Gassendi	3.6×10^3	5.4×10^3	3.0×10^4
Agrippa	1×10^4	7.3×10^3	4.0×10^3
Proclus	4.4×10^4	3.6×10^4	1.4×10^4

established in laboratory experiments²⁰ and possibly on the lunar surface²². The interaction between the solar wind and an emitted He-Ar cloud should exhibit this effect, although there are uncertainties due to scaling from the laboratory experiments to the lunar situation¹⁶. The supermagnetosonic plasma-neutral interaction should be characterised by the emission of radiation, primarily at infrared and longer wavelengths, which is partially plane polarised relative to the ambient magnetic field²³. This polarisation is a result of the nonlinear plasma processes in the interaction which create a population of nonthermal electrons in the interaction region with velocities primarily along the magnetic field direction. Unlike other proposed energy sources for LTP, this critical velocity model relies on internal lunar energy for the phenomena. The thermal energy of the escaping neutral gas is converted into free electron energy by the plasma interaction, and then into radiation by coulomb excitation of neutrals. That is, the magnetised solar wind acts like an electromagnetic catalyst which converts the expansion energy of the gas cloud into visible photons.

In addition to the other experiments¹³ which should be included on a lunar survey mission, a POLO spacecraft should carry instruments to detect and analyse the sporadic venting of lunar radiogenic gases. These should include: an α -particle spectrometer³ to measure the ²²²Rn and ²¹⁰Po distributions on the lunar surface²³ and thus indicate regions where recent venting has taken place⁴; a combined infrared spectrometer/polarimeter to determine the surface mineralogy by reflectance spectra^{24,25} and to search for the He and Ar emission lines expected in the plasma-neutral interactions^{16,26,27}; and a low-to-medium energy charged particle detector capable of both high spatial (pitch angle) and energy resolution. The last instrument could map the lunar surface remanent magnetic fields using the electron reflection technique^{28,29}, and could search for bursts of low energy (80–100 eV)^{26,27}, low pitch-angle (relative to the solar wind magnetic field) nonthermal electrons generated in gas clouds vented from the Moon by the proposed critical velocity effect.

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Palaeomagnetic field strengths from sediments baked by lava flows of the Chaîne des Puys, France

MEASUREMENTS of ancient geomagnetic strength are of great interest but are much less common than measurements of direction. This is due partly to the scarcity of materials which are both well dated and suitable for study, and partly to the amount of time required to make measurements using presently available techniques. We report here three new results from baked sediments which have been dated by thermoluminescence (TL) or radiocarbon methods. One of them is early Holocene and the others are late Pleistocene (Table 1). The localities have been described by Huxtable *et al.*¹, from whom we obtained samples.

Laboratory measurements were performed on cubic specimens (23 mm edges, cut from the samples), using a 'Digico' magnetometer with an 80-mm diameter balanced fluxgate detector⁵. Directions of natural remanent magnetisation (NRM) from those samples which were orientated have been discussed elsewhere¹.

Ancient field strengths were determined using the modified Thelliers' technique^{6–8}, which involves reheating specimens to progressively higher temperatures and comparing the ancient magnetisation with a new one acquired in a known laboratory magnetic field. Two heatings were performed at each temperature, using a furnace of the type described by Barbetti⁹ inside the Rubens coil system constructed by Weaver¹⁰. The first heating and cooling cycle at each temperature was performed in zero magnetic field, and the second in a magnetic field of 48.5 μ T. Measurements of remanent magnetisation were made at room temperature after every heating; values of the partial NRM (PNRM) remaining were obtained from the first of each pair of measurements, and values for the newly acquired partial thermoremanent magnetisation (PTRM) were obtained by vector subtraction between the pairs of measurements for each heating temperature.

The measurements were analysed in the normal way by constructing an NRM-TRM diagram, in which PNRM values are plotted against PTRM values with heating temperature as a parameter^{7,8}. Lines were fitted to appropriate sets of points using the 'maximum likelihood' method¹¹, assuming that errors in the PNRM and PTRM are normally and identically distributed but uncorrelated. The slopes of the fitted lines are a measure of the ratio of NRM to TRM, and estimates of ancient field strength are derived on the basis that the magnetisations are proportional to the magnetic fields in which they were acquired.

The results are summarised in Table 1, and representative NRM-TRM diagrams are shown in Fig. 1. Decisions about which points to include in the line-fitting analyses were made in an empirical fashion. Trial analyses were performed for each specimen and the high-temperature points progressively excluded until the width of the calculated confidence limits approached a minimum; final choices were made for each site after inspection of the results from the appropriate group of specimens.

Five of the St Saturnin specimens (from three black-coloured samples) gave consistent (though somewhat variable) results with a mean of $79 \pm 14 \mu$ T. Two specimens from sample i15 gave much lower values (Table 1 and Fig. 1), and have therefore been excluded from the mean. The low values might be explained by deformation or the introduction of small quantities of fine-grained material after the ancient baking, or they may be related to some peculiarity in the magnetic mineralogy.

Five red-coloured specimens from Royat gave highly consistent results (Table 1). The NRM-TRM diagrams (see Fig. 1) exhibited linearity over wide temperature ranges, with small departures (decreases in PTRM-carrying capacity) above 600 °C; they rank among the best we have ever obtained from

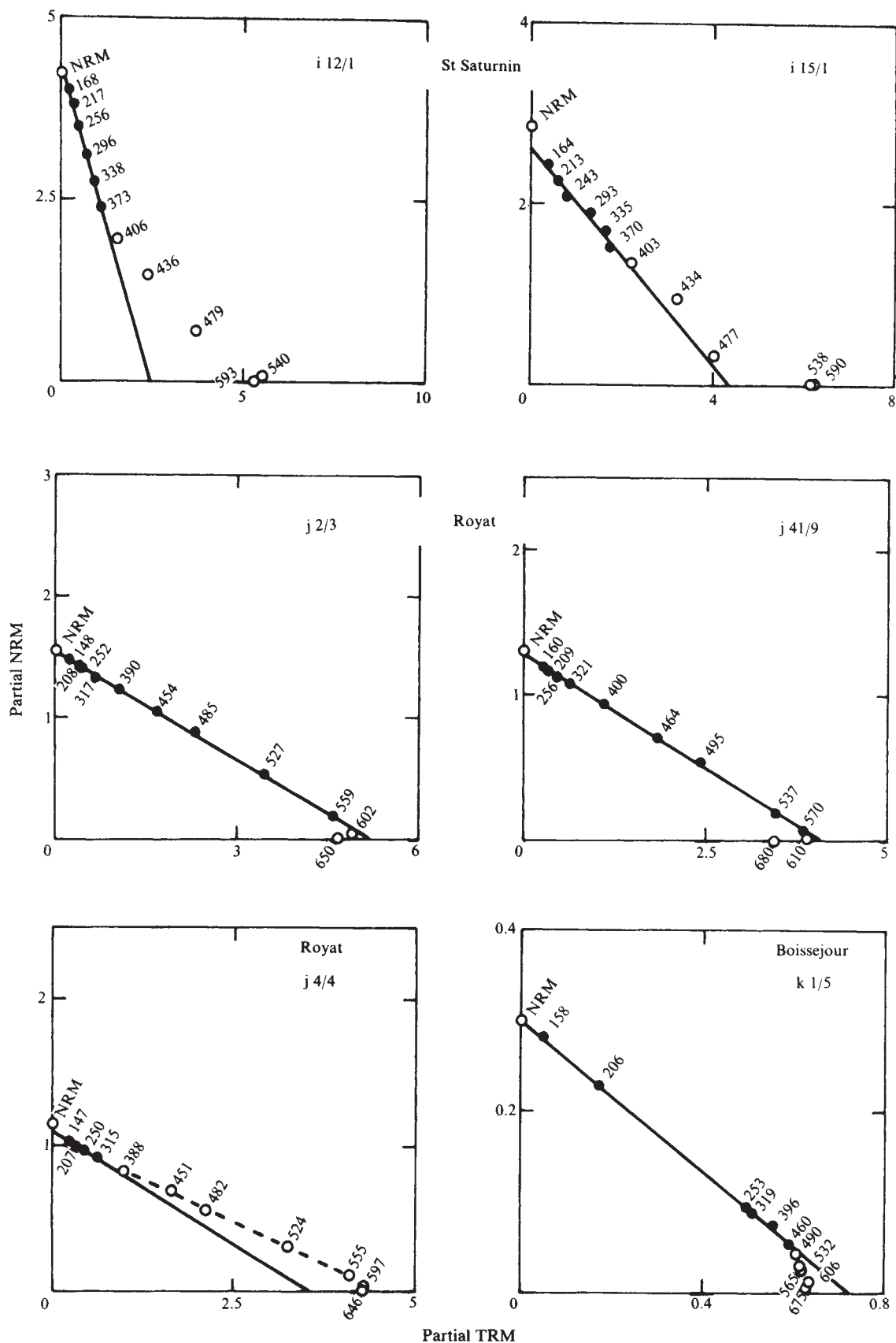


Fig. 1 Representative NRM-TRM diagrams. The units are $\text{mA m}^2 \text{ kg}^{-1}$, the horizontal scales are twice the vertical ones, and numbers near the points give the laboratory heating temperatures in $^{\circ}\text{C}$. Lines have been fitted to the sets of points represented by solid symbols. The dashed line for Royat specimen j4/4 has been fitted to the points from 388°C to 555°C ; it has a slope $\sim 25\%$ lower than the solid line and is thought to result from the introduction of new sediment after the ancient baking.

ancient materials. We also analysed specimen j4/4, from a separate sample which contained a small ($\sim 1\%$) but clearly visible proportion of grey-green clay distributed along internal fracture surfaces. Four points on the NRM-TRM diagram (Fig. 1) between 147°C and 315°C gave a result (Table 1) identical to the mean value of the other specimens, whereas the points from 388 to 555°C lie on a well defined line with significantly lower

slope. These observations agree with what one might reasonably expect; that the introduction of sediment after the ancient baking should make little difference to the PNRM but a great deal of difference to the PTRM, particularly at temperatures above $\sim 300^{\circ}\text{C}$ when minerals such as goethite may be transformed to haematite¹².

The four red-coloured specimens from Boissejour gave very

Table 1 Summary of ages and palaeomagnetic field strength measurements

Site	Age (yr BP)	Specimen	Temperature range (°C) (<i>n</i> points)	Ancient field strength (μT)	Dipole moment (10 ²² A m ²)
St Saturnin	8100 ± 800 OxTL 132i Ref. 1 7650 ± 350 Conventional ¹⁴ C Sa-90, Ref. 2	i12/1	168–373 (6)	85 ± 6	12.8 ± 4.2
		i12/2	162–356 (6)	83 ± 21	
		i17/1	162–368 (6)	91 ± 39	
		i19/1	162–368 (6)	71 ± 11	
		i19/2	166–456 (6)	63 ± 23	
			Mean	79 ± 14	
		i15/1	164–370 (6)	29 ± 7	
Royat	25,800 ± 2,200 OxTL132j Ref. 1	i15/2	172–376 (6)	39 ± 30	2.4 ± 0.2
		j2/3	148–559 (9)	14 ± 1	
		j2/4	146–544 (9)	16 ± 1	
		j41/5	134–558 (10)	15 ± 1	
		j41/9	160–570 (9)	15 ± 1	
		j41/24	146–551 (9)	15 ± 1	
		Mean		15 ± 1	
Boissejour	~ 50,000 OxTL132k Ref. 1	j4/4	147–315 (4)	15 ± 4	3.3 ± 0.9
		k1/5	158–460 (6)	20 ± 1	
		k1/6	156–457 (6)	20 ± 1	
		k1/9	156–455 (6)	20 ± 1	
		k1/14	156–449 (6)	21 ± 1	
		Mean		20 ± 1	

Provenance of the samples is given in ref. 1, specimens are identified by sample code and individual numerals after the slash. Column 4 gives the temperature range and number of points used in the final line-fitting analyses on the NRM-TRM diagrams. Estimates of ancient field strength are given with 95% confidence limits for each specimen, and site mean estimates are listed. Dipole moments have been calculated using the palaeomagnetic direction for Royat³ (to give a virtual dipole moment; ref. 4) and the axial dipole inclination for the other two sites (to give virtual axial dipole moments); the 95% confidence limits include contributions from the uncertainties in both strength and direction (α_{95} taken as 20° for St Saturnin and Boissejour).

similar results. An unusually large proportion of the total magnetisation (both NRM and TRM) was associated with temperatures below ~250 °C, suggesting the presence of a mineral (such as titanomagnetite) with a low Curie temperature in addition to the usual magnetic minerals with Curie temperatures above 550 °C. The specimens exhibited a noticeable decrease in PTRM-carrying capacity at temperatures above 460 °C (see Fig. 1), and the higher-temperature points have been excluded from the final line-fitting analysis.

Mean values of ancient field strength, and corresponding values for the geomagnetic dipole moment, are given in Table 1. These results have several implications. First, the value for St Saturnin is higher than the present-day dipole moment (8.0×10^{22} A m²). That confirms previous results obtained by Bucha^{13–15} from Çatal Hüyük in Turkey (mean 10.0×10^{22} A m²), but is somewhat higher than results from Japan¹⁶ (mean 6.1×10^{22} A m²). An average of those and the St Saturnin result yields a dipole moment of 9.6×10^{22} A m² for the period 8,200–7,000 conventional radiocarbon yr ago.

The virtual dipole moment for Royat (Table 1) is low and the palaeomagnetic direction (295.5°, +66.5° which is 26° from the axial dipole direction for the site) is also unusual³. The TL ages for Royat¹ and the Laschamp event ($33,000 \pm 4,000$ yr; refs 17, 18) are not significantly different, and they may be linked as previously suggested³. Also the Laschamp event may have occurred at the same time as the Lake Mungo geomagnetic excursion in Australia¹⁹ (which has a very similar TL age of $33,500 \pm 4,300$ yrs; ref. 20), and that the Royat flow may have occurred up to a few thousand years later (perhaps at the same time as the younger excursion at Lake Mungo when low values were recorded for dipole moment¹⁹). It is also interesting that a double-excursion occurred at around the same time at Mono Lake in California²¹.

The result for Boissejour (Table 1) indicates a low geomagnetic dipole moment at an earlier date. The possible effects of

geomagnetic strength variation on the radiocarbon time-scale for the late Pleistocene will be discussed in a forthcoming paper.

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Preferential formation of the atmosphere-sialic crust system from the upper mantle

In the past decade, extensive studies of the rare gas content of mantle derived rocks have led to several models of Earth degassing, all of which assume that the argon of the atmosphere degassed from the entire mantle. We give evidence here to support the concept of concurrent transfer of elements to the atmosphere and sialic crust preferentially from the upper mantle. This concept accounts for the following observations: (1) the ^{40}Ar concentrations in the rapidly quenched glassy margins of deep sea pillow basalts are too low for many estimates of the K concentration of the mantle^{1,2}; (2) the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of rocks derived from the deep mantle is similar to the atmospheric $^{40}\text{Ar}/^{36}\text{Ar}$ ratio³⁻⁷; (3) the upper mantle under oceanic ridges is depleted in K and other large-ion-lithophile elements. A schematic representation of the upper mantle depletion process is given in Fig. 1 which shows that partial melting associated with spreading ridges and Benioff zones exclusively in the upper mantle is responsible for the accretion of continents and associated degassing to the atmosphere.

The concentration of argon in glassy margins of submarine basalts provides evidence for depletion of argon in the upper mantle. Several factors can affect the composition and concentration of argon isotopes in submarine basalts, but degassing and contamination by atmospheric argon probably dominate⁸. The effect of atmospheric mixing is demonstrated in Fig. 2. In this type of plot, mixtures of atmospheric argon with a uniform source of mantle argon will form a line with a slope equal to ^{40}Ar content of the mantle and which connects the atmospheric and mantle $^{40}\text{Ar}/^{36}\text{Ar}$ values. Figure 2 shows basalt samples forming a broad mixing band between $^{40}\text{Ar}/^{36}\text{Ar}$ ratios above 10,000 and the atmospheric value of 295.5. The scatter in the data may be attributed to degassing which affects the $1/^{36}\text{Ar}$ values but

not the $^{40}\text{Ar}/^{36}\text{Ar}$ value. Concentration of argon into the melt during partial melting may contribute to the scatter, but probably to a lesser extent⁸. A maximum of $3.22 \times 10^{-6} \text{ ml g}^{-1}$ is derived for the ^{40}Ar concentration of the mantle source from the slope of the steepest mixing line in Fig. 2. We consider this value to be a maximum, not only because of the rather striking upper limit exhibited by the mixing line, but also because the ^{40}Ar concentration measured on what may be the least degassed oceanic ridge sample available, a 'popping rock' recovered from 5,750 m in the Cayman Trough, is $2.0 \times 10^{-6} \text{ ml g}^{-1}$, well below this value. Furthermore, 65% of all the ^{40}Ar concentrations measured form a distinctive peak between 0.9×10^{-6} and $3.0 \times 10^{-6} \text{ ml g}^{-1}$ with the other 35% of the data scattered between 0.9×10^{-7} and $0.9 \times 10^{-6} \text{ ml g}^{-1}$ (ref. 2).

The concentration of ^{40}Ar in the oceanic crust can be used to compute the original K content of the mantle by calculating the 'reconstituted' concentration of ^{40}Ar , that concentration of ^{40}Ar that would now exist in the mantle if it had never degassed ^{40}Ar to the atmosphere. Previously published estimates of reconstituted ^{40}Ar have assumed the atmosphere ^{40}Ar degassed from the entire mantle. In the preferential degassing model presented here, the mass of the mantle from which the atmosphere-sialic crust ^{40}Ar was derived, is allowed to vary from 0 to 100% of the total mantle and the reconstituted ^{40}Ar is calculated according to the formula

$$\Sigma^{40}\text{Ar} = [^{40}\text{Ar}_m] + \frac{^{40}\text{Ar}_{at} + ^{40}\text{Ar}_s}{xM} \quad (1)$$

where; $\Sigma^{40}\text{Ar}$ = the reconstituted ^{40}Ar , the concentration of ^{40}Ar that would exist in the mantle today after returning the ^{40}Ar from the atmosphere and sialic crust to the portion of the mantle from which it degassed; $[^{40}\text{Ar}_m]$ = the concentration of ^{40}Ar now in the depleted mantle, here represented by the ^{40}Ar in glassy submarine basalt; $^{40}\text{Ar}_{at}$ = the mass of ^{40}Ar in the atmosphere; $^{40}\text{Ar}_s$ = the mass of ^{40}Ar in the sialic crust, estimated by assuming that the K content of the sialic crust is 2.5% and the average argon retention age 2,000 Myr; x = the fraction of the mantle from which the atmosphere and sialic crust formed; M = the mass of the mantle ($42.56 \times 10^{26} \text{ g}$).

The original K content of the mantle can be calculated from ^{40}Ar assuming an age 4,500 Myr for the Earth. The results are depicted in Fig. 3, which plots $[^{40}\text{Ar}_m]$ versus the per cent of the mantle degassed. As shown in Fig. 3, if the ^{40}Ar content of glassy submarine basalts represents the maximum ^{40}Ar of the depleted mantle today, and the atmosphere degassed from the entire mantle, then the original K content of the mantle was <200 p.p.m. An original K content of the Earth this low is

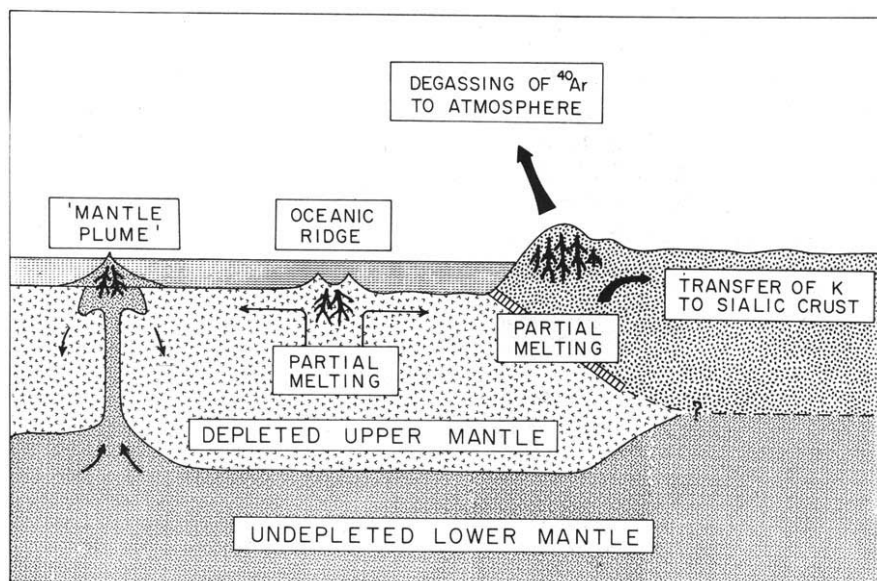


Fig. 1 Diagrammatic representation of preferential formation of the atmosphere and sialic crust from the upper mantle.

incompatible with many whole-Earth heat flow models. Higher values for the original K content are allowed if the atmosphere and sialic crust degassed preferentially from the upper mantle.

Further implications of the concept of preferential degassing of the atmosphere can be demonstrated by constructing a model of Ar and K distribution in the Earth based on the following constraints: (1) the atmosphere-sialic crust system formed from the upper 415 km of the mantle. (2) Degassing of ^{40}Ar took place, with simultaneous transfer of K to the sialic crust⁹. (3) The maximum ^{40}Ar of the depleted upper mantle is equivalent to the maximum ^{40}Ar in glassy submarine basalts (see Fig. 2).

The first constraint limits the zone of preferential depletion to the upper 415 km, or about 25% of the total mantle. The 415-km seismic step depth was chosen because of a seismic velocity increase which corresponds to a transition from a zone of low density to a zone of high density and may represent a transition from depleted to non-depleted mantle. If it is assumed that all the atmospheric ^{36}Ar degassed from the depleted zone, it is possible to calculate that the ^{36}Ar of the non-depleted mantle is $\sim 10^{-7} \text{ ml g}^{-1}$. The second constraint specifying coherency between argon degassing and K transfer is justified because all of the ^{40}Ar of the atmosphere can be produced from a sialic crust containing a 2.5% K (ref. 9). The actual value of K may be as low as 1.25%, therefore the amount of K which must be depleted from the upper 415 km of the mantle to produce the sialic crust ranges from 240 to 475 p.p.m. Constraint (3) permits calculation of ^{40}Ar of the non-depleted lower mantle (see equation (1)), which for preferential degassing to 415 km is calculated to be $4.5 \times 10^{-5} \text{ ml g}^{-1}$. The $^{40}\text{Ar}/^{36}\text{Ar}$ of the non-depleted mantle ($4.5 \times 10^{-5}/1.0 \times 10^{-7}$) is 450. The original K content of the non-depleted mantle is calculated from $\Sigma^{40}\text{Ar}$ and the stipulation of degassing to 415 km and is found to be 660 p.p.m. (Fig. 3). By subtracting the K removed to the sialic crust, the K content of the depleted mantle is calculated to be 125–422 p.p.m.

All the results of the model calculations are summarised in Table 1. This model is not presented as a unique solution for the preferentially degassed mantle, but rather as a starting matrix

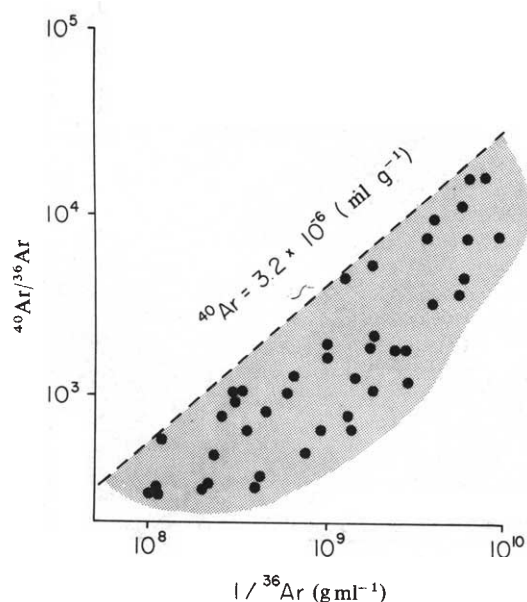


Fig. 2 The $^{40}\text{Ar}/^{36}\text{Ar}$ ratio compared with the $1/^{36}\text{Ar}$ ratio in glassy submarine basalts. This plot is used to demonstrate the effects of mixing atmospheric argon ($^{40}\text{Ar}/^{36}\text{Ar} = 295$) with mantle argon of high $^{40}\text{Ar}/^{36}\text{Ar}$. The dashed line probably represents the mixing line of steepest slope and may correspond to the maximum ^{40}Ar of the oceanic crust. The data are from refs 8, 15–17 and unpublished.

from which more sophisticated solutions can evolve. Several possible perturbations on the model are envisaged.

First, loss of ^{40}Ar to the atmosphere from upper mantle without simultaneous transfer of K to the sialic crust would increase the K content of the depleted upper mantle while decreasing the K content of the sialic crust, but would not change the K content of the non-depleted mantle.

Second, modification of the thickness of the zone of depletion can decrease or increase possible solutions for the K and Ar contents of the mantle, but would not change the solutions for the K content of the sialic crust.

Third, if the K concentration of the sialic crust is less than 2.5%, a higher percentage of ^{40}Ar must have degassed directly from the mantle without coherent formation of the sialic crust. This would result in higher K concentrations for the depleted mantle. Furthermore, the ^{40}Ar of the sialic crust would be lower than the value used in this model to calculate $\Sigma^{40}\text{Ar}$ and would result in lower solutions for the K content of the non-depleted mantle. For example, if the K concentration of the sialic crust is 1% instead of 2.5% as used in the model calculation, $\Sigma^{40}\text{Ar}$ and K concentration of the non-depleted mantle would be 13% lower.

Several implications of the idea of preferential degassing can be tested by comparing data on samples thought to represent the

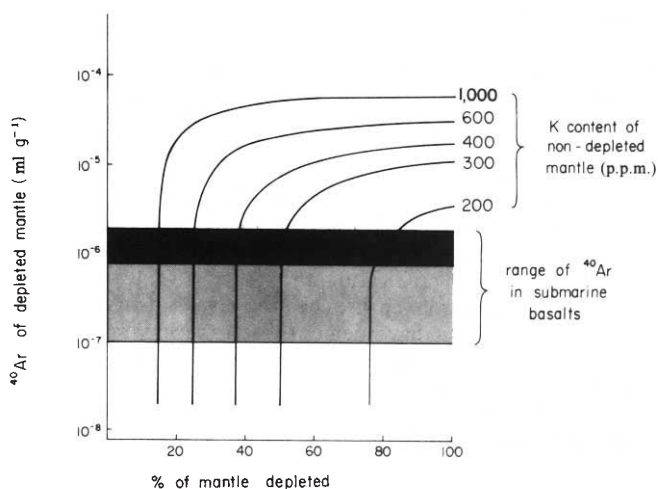


Fig. 3 The ^{40}Ar content of the present day upper mantle compared with the per cent of the mantle from which the atmosphere-sialic crust formed. The iso-K curves represent solutions of the initial K content of the mantle derived by recombining the atmospheric-sialic component of ^{40}Ar with the ^{40}Ar presently in the depleted portion of the mantle.

depleted and non-depleted mantles. Ocean ridge basalt probably originated in the depleted zone, while basalts associated with 'plumes' and many continental ultramafics may originate in the deep mantle. The most reliable samples of deep mantle may be continental ultramafic rocks containing anomalous ^3He , ^{21}Ne , or ^{129}Xe or exhibiting a 'planetary' type rare gas abundance pattern. The planetary-type rare gas abundance pattern is characterised by a high $^{84}\text{Kr}/^{130}\text{Xe}$ ratio compared with the atmosphere ratio and a low $^{20}\text{Ne}/^{36}\text{Ar}$ ratio compared with the ratio in oceanic ridge basalts¹⁰. Deep mantle-derived samples include: a kaersutite inclusion in nephelinitic basalt from New Zealand containing excess ^3He and ^{21}Ne and exhibiting a planetary rare gas abundance pattern³; josephinite, a rare terrestrial nickel-iron alloy containing elemental silicon and thought by some to have originated from the core-mantle boundary and containing excess ^3He , ^{21}Ne , and ^{129}Xe ^{6,7}; a kearsutite inclusion in alkali basalt from Dish Hill, California, containing excess ^3He and exhibiting a planetary type rare gas abundance pattern⁵; two olivine megacrysts from South African

kimberlite pipes containing excess ^3He and ^{129}Xe and a planetary rare gas abundance pattern and samples of kimberlite, carbonatite and diamond from South Africa^{5,11}.

The model calculations imply that the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of the deep mantle is similar to that of atmosphere (see Table 1). This stems from the assumptions that the deep mantle has remained a closed system and the atmosphere now contains more than 80% of all the ^{40}Ar and more than 99% of the ^{36}Ar that would have currently been in the upper mantle if it had remained a closed system. It is also important that according to the model calculations the upper mantle has been depleted in ^{36}Ar by a factor of >100 whereas it has been depleted in K by a factor not greater than 6; the net result being, that $^{40}\text{Ar}/^{36}\text{Ar}$ ratios as high as 16,000 can be expected in the depleted upper mantle. A comparison of the available data with the model is summarised in Fig. 4 which is a histogram comparing the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in basalts dredged from plume areas and continental ultramafics uncontaminated by crustal material, with submarine ridge basalts. The $^{40}\text{Ar}/^{36}\text{Ar}$ of the plume basalts and ultramafics range from 295 to 1,200 with the majority falling below 500, while ridge basalts range up to 16,000 with the majority falling above 1,000. It is interesting that, all continental ultramafics containing ^3He , ^{21}Ne , or ^{129}Xe , have a $^{40}\text{Ar}/^{36}\text{Ar}$ ratio below 850. This observation has led several workers to suggest that the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of the mantle source is similar to that of atmosphere³⁻⁵. The possibility of a mantle with a low $^{40}\text{Ar}/^{36}\text{Ar}$ ratio was first proposed on the basis of the initial ratio derived from K-Ar isochron plots of basalts and dolerites¹², although these samples may have been contaminated by atmospheric or crustal argon. For deep mantle ultramafics which have excess ^3He and relatively low $^{40}\text{Ar}/^{36}\text{Ar}$ ratios, crustal contamination can be ruled out because of the high concentrations of ^4He in crustal rock. On the other hand, the high concentration of ^{36}Ar in the atmosphere makes it conceivable that atmospheric contamination after extrusion has produced the low $^{40}\text{Ar}/^{36}\text{Ar}$ ratios without completely eradicating the other rare gas isotopic anomalies. However, stepwise heating experiments on low $^{40}\text{Ar}/^{36}\text{Ar}$ ultramafics have demonstrated the low ratio exists even in the high temperature fractions which are least likely to be contaminated by atmospheric argon⁴.

The model presented above also requires that the deep-mantle samples have higher rare gas contents than upper mantle samples. The evaluation of this implication from existing data is difficult because crustal contamination and degassing may have altered the concentrations. The ^{36}Ar concentration in the glassy margins of oceanic basalts is variable, but after accounting for the effects of atmospheric contamination and degassing, the ^{36}Ar concentration of the upper mantle under oceanic ridges has been estimated to be $<2 \times 10^{-10} \text{ ml g}^{-1}$ (ref. 1). With the exception of diamonds, the available ^{36}Ar data on the continental ultramafic samples used in Fig. 4 fall in the range $0.38\text{--}3.6 \times 10^{-8} \text{ ml g}^{-1}$, distinctly higher than estimates of the ^{36}Ar in the upper mantle under the oceanic ridges. The high abundance of ^{36}Ar in these samples may reflect the ability of these minerals to preferentially accommodate rare gases in their structure relative to other phases in the mantle and does not prove that the deep

mantle has a higher concentration of rare gases than the upper mantle. Nonetheless, the data are consistent with and support the model of preferential degassing of the upper mantle. The only ultramafic samples known at present with anomalous ^3He or ^{129}Xe , which have $^{40}\text{Ar}/^{36}\text{Ar}$ ratios $>1,000$, are ultramafic xenoliths from Hawaii^{12,13}. This observation may reflect the possibility that the mantle is more extensively depleted in oceanic regions than under the continents, or that these xenoliths originated in the depleted upper mantle.

The concept of preferential formation of the atmosphere and sialic crust from the upper mantle provides a means of depleting K and other incompatible elements in the mantle beneath oceanic ridges and is consistent with rare gas data on mantle derived rocks. It is especially useful in accounting for possible similarities in the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of the atmosphere and rocks of deep mantle origin.

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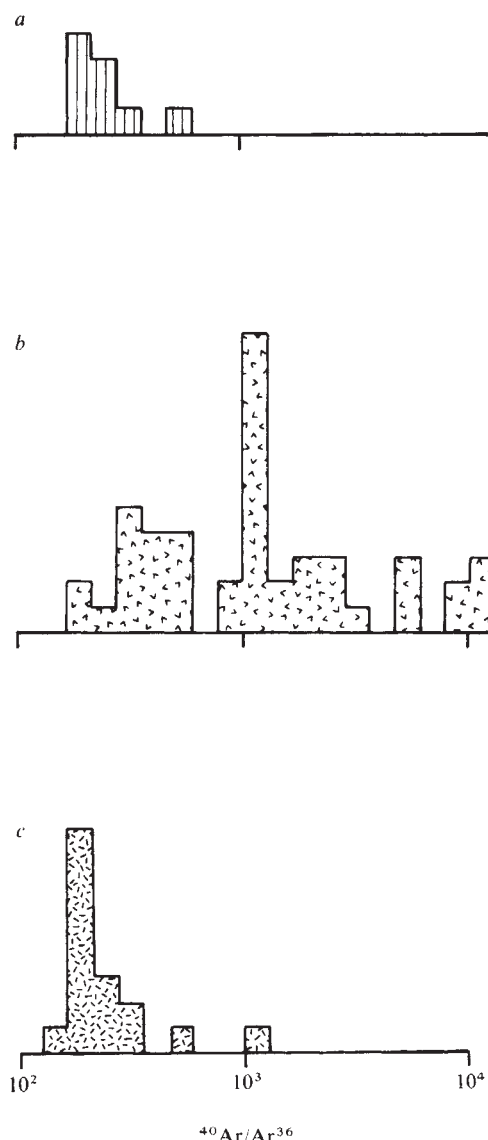


Fig. 4 A histogram comparing the $^{40}\text{Ar}/^{36}\text{Ar}$ ratios of: a, continental ultramafics exhibiting ^3He , ^{21}Ne , and ^{129}Xe anomalies; b, glassy submarine basalts from oceanic ridges; and c, glassy submarine basalts from plume areas. Data are from refs 4-8, 11, 15-17 and unpublished.

Table 1 Model calculations of the K and Ar concentrations in major units of the Earth*

	K (p.p.m.)	^{40}Ar ($10^{-6} \text{ ml g}^{-1}$)	^{36}Ar ($10^{-8} \text{ ml g}^{-1}$)	$^{40}\text{Ar}/^{36}\text{Ar}$
Atmosphere	—	9.08†	3.37†	295.5
Sialic crust	12,500-25,000	175-350	0.50-2.00‡	8,750-70,000
Upper mantle	125-422	3.20§	0.02-0.04§	8,000-16,000
Lower mantle	660	45	10.00	450

*See model constraints in text.

†Total argon of atmosphere is divided by the mass of the mantle.

‡The ^{36}Ar content of the sialic crust is estimated from the range of concentrations in crustal rocks¹.

§Estimated from glassy submarine basalts¹.

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Sapphirine-bearing rocks with sedimentary and volcanogenic protoliths from the Arunta Block

At least 15 separate localities for sapphirine-bearing rocks have been found in the Arunta Block, a Precambrian metamorphic complex in central Australia (Fig. 1), although only two have been documented in any detail¹⁻³. Such rocks are the most spectacular examples of a suite of rocks with a distinctive, characteristic chemistry. This suite includes, *inter alia*, the better known cordierite-anthophyllite rocks, and is widely distributed both within the Arunta Block and world-wide. From field observations it is proposed here that two different geological processes, one sedimentary or diagenetic and the other volcanic, generated rocks that are now indistinguishable in their whole-rock chemistry and mineral assemblages. It had formerly been proposed that sapphirine-bearing rocks of volcanogenic and sedimentary origins were mineralogically and chemically distinct⁴.

Rocks in this chemical suite have compositions in which >90% of the oxide total is contributed by SiO₂, Al₂O₃, MgO, and FeO; representatives are either silica-undersaturated or contain only small quantities of modal quartz. Metamorphic grade affects the proportion of modal quartz for a given SiO₂ content, as well as the other minerals which may include talc, chlorite, phlogopite-biotite, anthophyllite, gedrite, cummingtonite, cordierite, sillimanite, kyanite, corundum, staurolite, orthopyroxene, kornorupine (boron-bearing), garnet, sapphirine and spinel. These minerals and whole-rock compositions may be shown diagrammatically on a quaternary SiO₂, Al₂O₃, and MgO + FeO plot (Fig. 2). Such a plot cannot take account of the effect of MgO/FeO on mineral assemblages: staurolite and garnet may occur if MgO/FeO (whole-rock composition) is low, whereas sapphirine requires high MgO/FeO. The effects of metamorphic facies and variations within the chemical constraints are shown in Table 1, using examples from various localities within the Arunta Block.

Whole-rock analyses from the chemical suite define a trend slightly more aluminous than the tie quartz-chlorite in Fig. 2, as do estimates of composition derived from mineral assemblages. This agrees substantially with previously published statements^{5-7,14} that some cordierite-anthophyllite rocks originated as chlorite-rich rocks, but implies an additional aluminosilicate component, such as kaolinite, illite, phengite, muscovite or feldspar. Sapphirine-bearing rocks (and hypersthene-garnet-bearing rocks), being higher metamorphic facies equivalents of cordierite-anthophyllite rocks, may have similar origins. Figure 2 also shows that even apparently 'sedimentary-type' sapphirine-bearing rocks could not have originated as mixtures of clay minerals, although for such rocks magnesite-rich protoliths should be considered.

On the basis of geological setting two major groups of rocks belonging to the chemical suite are recognised in the Arunta Block.

Group 1: sedimentary type. In the Reynolds Range sapphirine-bearing and chemically akin rocks crop out intermittently over a kilometre along the (regional) unconformity between an eroded sequence of pelites and an overlying quartzite⁸. Similar rocks, also sapphirine-bearing, crop out in the same stratigraphic position, some 10 km to the east, and similar, though slightly more siliceous, rocks crop out on the same unconformity several kilometres to the south. A hundred kilometres to the east, near Delmore Downs, specimens of quartz-chlorite-anthophyllite-cordierite granofels have been collected at the same regional unconformity. In the Reynolds Range a pod of sapphirine-anthophyllite-cordierite-enstatite granofels with cordierite-rich granofels also occurs several hundred metres above the unconformity within a pelitic sequence.

The geological processes that generated these magnesian minerals must have been compatible with the deposition and diagenesis of the enclosing non-volcanic sedimentary sequences. A possible protolith would be a mixture of magnesite, a clay (illite or kaolinite), haematite and quartz (see ref. 9) formed by surface waters with high pH and CO₂ content. An alternative origin might have been by way of meerschaum or sepiolite deposits generated from alkaline groundwaters¹⁰. As these rocks are low in CaO, they are not metamorphosed marls, unlike the sapphirine-bearing rocks of southern Madagascar¹¹. These rocks contain appreciable FeO, and include examples lower in SiO₂ when compared to the chemically similar white schists of sedimentary origin in Zambia and Afghanistan¹².

Group 2: volcanic type. Rocks belonging to the chemical suite are also widely distributed in the granulite terrain of the Harts and Strangways Ranges within metamorphosed volcanic and volcanoclastic sequences of acid and basic composition¹³. Occurring as pods and layers associated with distinctive cordierite quartz-rich granofels, they are subdivided on field setting into two subclasses. Those with discordant relationships originated as chlorite-rich pods within zones of extensive chloritic alteration cross-cutting the original volcanic stratigraphy. Others occur as a concordant basal layer in a three-tier

Table 1 Assemblages in examples of the chemical suite selected from the Arunta Block

Chemical control		Prograde		Retrograde	
High SiO ₂	High MgO	Qz-Cd-Opx	RR	Qz-Ky-Cd-Ged	EC
	High FeO	Qz-Cd-Hy ⁺ Si±Ga	EC, MM	Qz-Anth-Cd-Ch	EC
		Qz-Ch-Anth-Cd±Ga	DD		
Low SiO ₂	High MgO	Cor-Sp-Sap-Si-Hy-Cd	MM		
		En-Cd-Sap-Ph	RR		
	High FeO	Sp-Ga-Hy-Cd	MM, EC	Cor-St-Ch	EC
				St-Ga-Ch	EC

Minerals: Anth, Anthophyllite; Cd, Cordierite; Ch, Chlorite; Cor, Corundum; En, Enstatite; Ga, Garnet; Ged, Gedrite; Hy, Hypersthene; Ky, Kyanite; Opx, Orthopyroxene; Qz, Quartz; Si, Sillimanite; St, Staurolite; Ph, Phlogopite; Sp, Spinel.

Localities (see Fig. 1): DD, Delmore Downs; EC, Edwards Creek; MM, Mount Milton; RR, Reynolds Range.

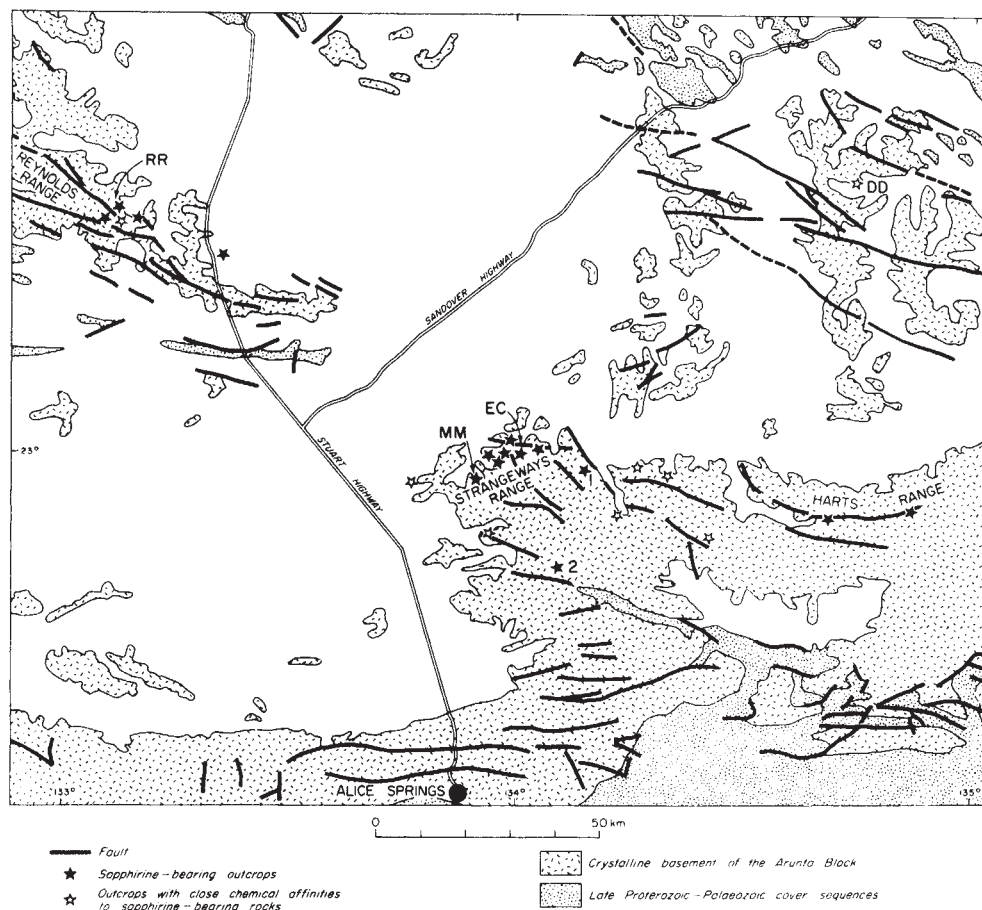


Fig. 1 Distribution of sapphirine-bearing outcrops, and outcrops of chemically allied rocks in the Arunta Block, central Australia. Localities referred to in text: RR, Reynolds Range; DD, Delmore Downs; MM, Mount Milton; EC, Edwards Creek. 1, Woodford and Wilson (1976); 2, Hudson and Wilson (1966).

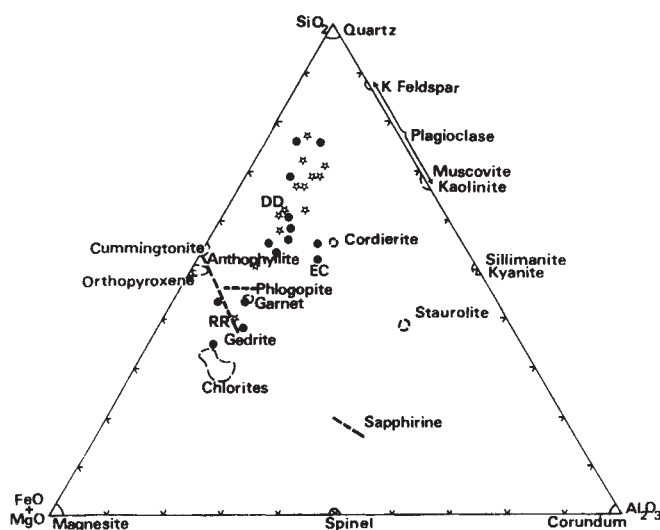


Fig. 2 Ternary plot into plane molar SiO_2 , Al_2O_3 , $\text{MgO} + \text{FeO}$, showing both minerals characteristic of the 'chemical suite' and also whole rock compositions, from the literature and from the Arunta Block as indicated. Stars indicate sedimentary origin, dots volcanogenic origins. Note overlap of fields.

association with impure marbles and/or calc-silicate granofels and banded gneisses consisting of quartz and iron minerals (magnetite, grunerite, and so on). These are interpreted as metamorphosed distal deposits from exhalative activity (Fig. 3). At some localities both subclasses crop out in close proximity; elsewhere only one type is present. This interpretation is similar to that proposed for some cordierite-anthophyllite rocks in the Canadian Shield^{6,14}, an interpretation enhanced by the presence of pyrite, galena, sphalerite, and chalcopyrite in many of the layered outcrops¹⁵. Sapphirine-bearing outcrops in the Yilgarn Shield have been interpreted as altered mafic volcanic rocks¹⁶. Relatively unmetamorphosed per-magnesian chloritic rocks of volcanic origin are rare but are known, for example, from the Woodlawn orebody in southeastern Australia¹⁷. Previously sedimentary origins have been proposed for some sapphirine-bearing (and chemically related) rocks in the Strangways Range^{3,18}.

Only field relationships are presently useful in distinguishing the two groups. Chemical analyses (from the Arunta Block and elsewhere) of the two types show such an overlap (see Fig. 2) that they provide no obvious discriminants.

Several outcrops shown in Fig. 1 were discovered by other members of a project team from the Bureau of Mineral Resources studying the Arunta Block. Constructive criticism and discussion proffered by colleagues at BMR and the Northern Territory Geological Survey, and by B. J. Hensen, N. Herriman, D. F. Sangster, W. Schreyer, and R. H. Vernon is

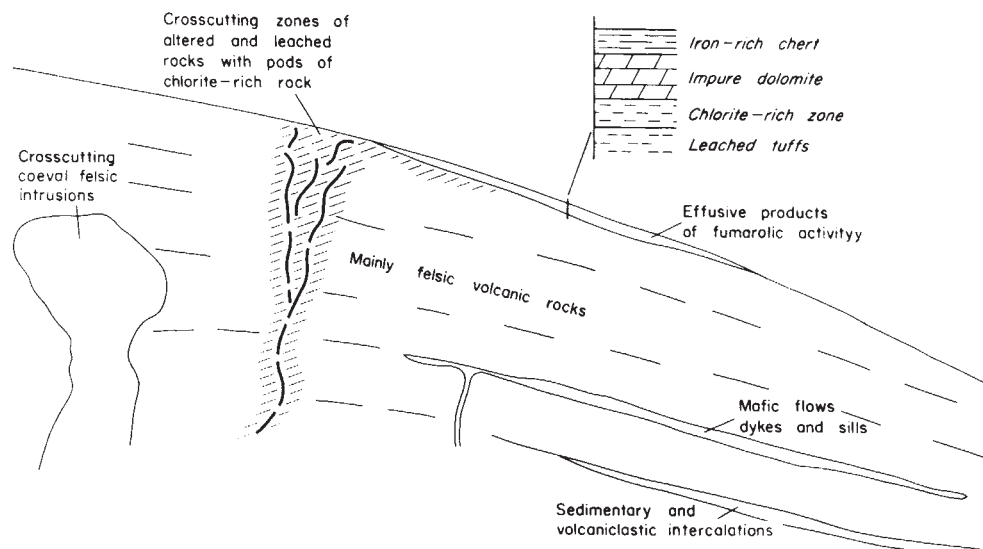


Fig. 3 Inferred settings for the formation of protoliths to sapphirine-bearing rocks (and the chemically allied rocks) in the Strangways Range.

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Sediment loads in the Amazon River

ESTIMATES published in the past 20 years of the sediment load delivered to the sea by the Amazon River range from 4 to 10×10^8 tonnes yr^{-1} . Estimates published around 1960, when few data were available on either the sediment concentration or the river discharge, were in the range $9\text{--}10 \times 10^8$ tonnes yr^{-1} (refs 1, 2). Estimates published in 1967 and 1968, after significant new data had been collected, were in the lower range of $4\text{--}5 \times 10^8$ tonnes yr^{-1} (refs 3, 4). Our more comprehensive data collected mostly since 1970 (and especially in 1977) suggest that the earlier higher estimates may have been more nearly correct. We show here that the mean annual load of suspended sediment at Óbidos, Brazil is between 8 and 9×10^8 tonnes yr^{-1} . Most of this sediment is discharged onto the continental shelf.

During the high-water seasons of 1976 and 1977, we measured sediment loads in the Amazon River basin. Our most

complete set of measurements were made during May and June of 1977, when we followed the annual high-water stage down the Amazon and measured the suspended-sediment loads at six places on the mainstem and in the two largest tributaries (Fig. 1). Because our ship moved downriver at about the same speed as the flood wave (considering the sampling stops we made en route), we came close to following the same relative flood stage for about 3,000 km from Peru to near the mouth of the Amazon. This gave us added insight into how the sediment load changed downriver.

The amounts of sediment being carried in suspension in 1977 were measured in samples of river water collected from RV Alpha Helix with a large-volume, depth-integrating sampler. The depth-integrating sampler is lowered to the river bed and raised back to the surface at a uniform vertical rate; its intake nozzle is designed to admit the sediment–water mixture at stream velocity. The resulting sample is thereby weighted for differences in velocity in different parts of the water column. One only need multiply the concentration of sediment measured in such a sample by the corresponding water discharge to obtain the suspended-sediment load^{5,6}. At each of the locations listed in Table 1, we took depth-integrated samples at three to seven stations spaced more or less evenly across the width of the river. Further information on our sampling and laboratory procedures, as well as a complete tabulation of the suspended-sediment data, are published elsewhere⁷.

Water discharges reported in Table 1 for the five mainstem sections in Brazil were estimated from the stage–discharge relationships (rating curves) that have been developed for these stations by Hidrologia S.A. and Companhia de Pesquisa de Recursos Minerais (CPRM). River stage is measured daily and river discharge is measured monthly or bimonthly at these stations. At Iquitos, where no independent measurements were available, we made a rough calculation of water discharge from our velocity data and the area of the cross-section measured by sonar (depth) and radar (width). The discharge of the Rio Madeira is estimated from measurements that Hidrologia S.A. makes monthly at its furthest downstream gaging station on the Madeira, about 200 km upriver of where we collected samples of suspended sediment. Discharge of Rio Negro, whose flow is not measured, was estimated from the difference between discharge at Óbidos and at other measured and unmeasured tributaries as detailed in the footnote to Table 1.

Suspended-sediment loads remain fairly constant in the mainstem between Iquitos and Manacapuru, indicating that the sediment in this part of the river is derived from the Andes while the jungle-draining tributaries that enter the river in Brazil bring in large amounts of water but little additional sediment. This

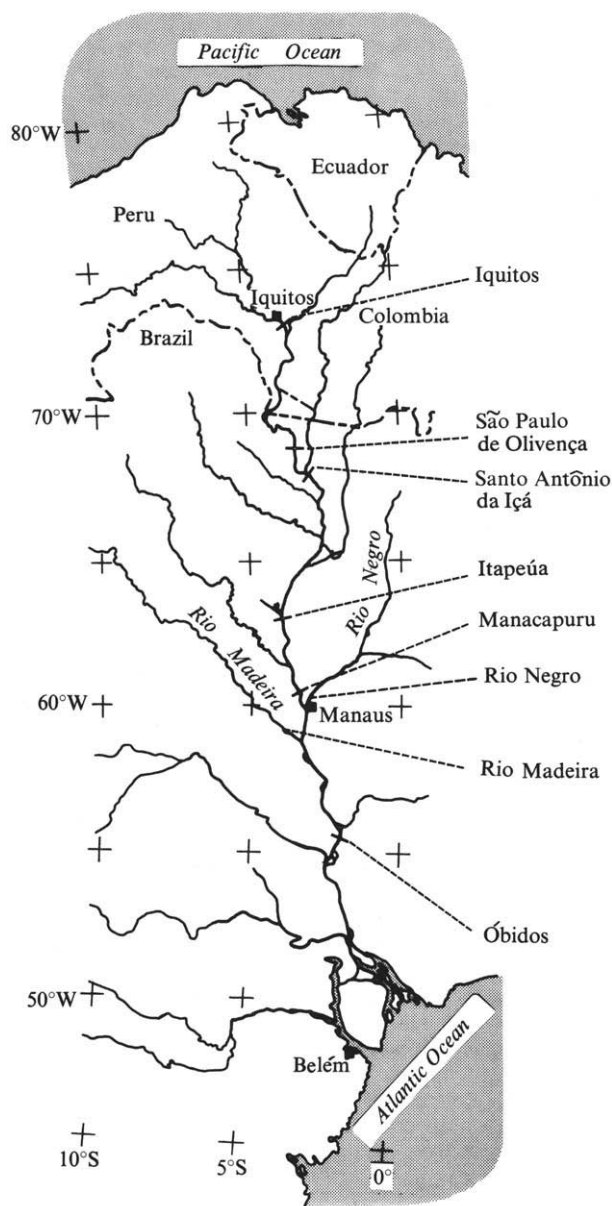


Fig. 1 Map of Amazon River, showing locations on mainstem and tributaries where sediment loads were measured in 1977.

feature was noted earlier by Sioli⁸ and was elucidated by Gibbs³, who estimated that 82% of the suspended solids discharged by the river were derived from the 12% of the total area of the Amazon basin that lies in the mountainous terrain of the Andes. The epitome of the jungle tributaries is Rio Negro which contributes ~20% of the water flow of the total Amazon system but only a trivial proportion of the sediment. Rio Madeira, on the other hand, has its headwaters in the Andes of Bolivia, and it carries a significant load of sediment.

The downstream decrease in the discharge-weighted concentrations shown in Table 1 was noted earlier by Gibbs³, but our data disagree with his in several places (Fig. 2). We were unable, in either 1976 or 1977, to detect the concentrations $>400 \text{ mg l}^{-1}$ that Gibbs reported during high-water seasons in the upper river. At Óbidos in the lower river, we measured higher concentrations than he did. After discussions with Gibbs we agree that the differences between our data and his are probably related to differences in sampling procedure. However, this does not rule out the possibility that the differences are real differences between one year and another.

Because the data in Table 1 represent only a single day's sediment load near the annual peak of river flow, we cannot extrapolate the figures by themselves to obtain long-term loads. However, by combining our sediment data with other unpublished data on the annual cycle of river discharge and the concentration of suspended sediment at low discharge, we can make a new estimate of the average annual load of suspended sediment at Óbidos. The section at Óbidos is the furthest downstream point on the mainstem at which river discharge is measured regularly.

The mean monthly discharges at Óbidos for a 4-yr period are shown in Fig. 3a. These averages were computed from the daily measurements of river stage and the less frequent (mostly bimonthly; some monthly) measurements of river discharge that were made in this period by CPRM and Hidrologia S.A. for the Departamento Nacional de Águas e Energia Elétrica (DNAEE).

The concentration of suspended sediment at low water was measured at Óbidos on 21 November 1963, by Ames of the

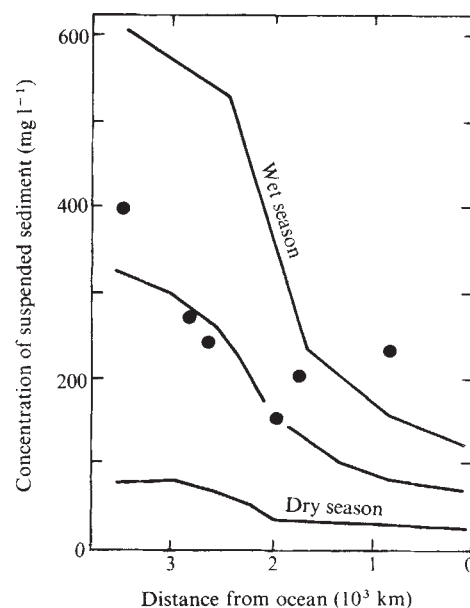


Fig. 2 Concentrations of suspended sediment in Amazon River mainstem. Solid lines represent concentrations reported by Gibbs³ for wet, intermediate, and dry seasons. Dark circles are the discharge-weighted concentrations listed in Table 1.

USGS during the first concerted efforts to measure the flow of the Amazon⁹. River discharge on that day was measured at $72,500 \text{ m}^3 \text{ s}^{-1}$. Ames collected 13 point samples of suspended sediment from different depths and at different locations across the river, and he calculated a discharge-weighted concentration of about 50 mg l^{-1} .

If we assume that our measurement of 235 mg l^{-1} represents the discharge-weighted concentration of suspended sediment at high discharge at Óbidos and that Ames' measurement of 50 mg l^{-1} represents the concentration at low discharge, and if we assume that the relationship between mean monthly discharge and mean monthly concentration follows the power relation plotted in Fig. 3b, we can compute monthly sediment loads for the average monthly discharges shown in Fig. 3a, and we can sum the monthly loads to obtain an estimate of the mean annual load of suspended sediment at Óbidos. This procedure gives an estimate of $9.3 \times 10^8 \text{ tonnes yr}^{-1}$. A slightly more conservative estimate can be computed if we assume that the mean concentration in the month of highest discharge is only 200 mg l^{-1} and that the concentrations in the other months range correspondingly between this value and the minimum

Table 1 Suspended-sediment loads measured in Amazon River and its tributaries, 1977

Location	Date	Water discharge ($\text{m}^3 \text{s}^{-1}$)	Suspended sediment	
			Concentration (mg l^{-1})	Load ($10^6 \text{ tonnes d}^{-1}$)
Iquitos	20 May	48,000	400	1.7
São Paulo de Olivença	22 May	70,000	275	1.7
Santo Antônio do Iça	23 May	80,000	245	1.7
Itapeúa (Coari)	26 May	110,000	150	1.4
Manacapuru	27 May	130,000	200	2.2
Rio Negro	28 May	50,000*	5	0.02
Rio Madeira	1 June	40,000	330	1.1
Óbidos	2 June	230,000	235	4.7

* Estimated by subtracting from discharge at Óbidos ($230,000 \text{ m}^3 \text{s}^{-1}$) the following: discharge of mainstem at Manacapuru (130,000), estimated discharge of Rio Madeira at Novo Aripuanã (40,000), and estimated discharge of Rio Trombetas (5,000) and other tributaries (5,000) between Manacapuru and Óbidos.

concentration of 50 mg l^{-1} ; in this case, the estimated annual suspended-sediment load at Óbidos is $8.2 \times 10^8 \text{ tonnes yr}^{-1}$.

How much of this sediment is discharged onto the continental shelf at the mouth of the river? Óbidos is about 850 km (river distance) above the mouth of the Amazon. The tributaries that enter the river below Óbidos add another 10% to the water discharge but do not add any significant amount of sediment. The question centres on how much of the sediment is deposited on the wide alluvial plain and in the extensive delta that lie between Óbidos and the sea. Even if this amount is substantial—as much as 20% of the sediment that passes Óbidos, for example—the remaining sediment load would place the Amazon behind only the Yellow River of China and the Ganges–Brahmaputra system of India and Pakistan as a supplier of sediment to the oceans⁴.

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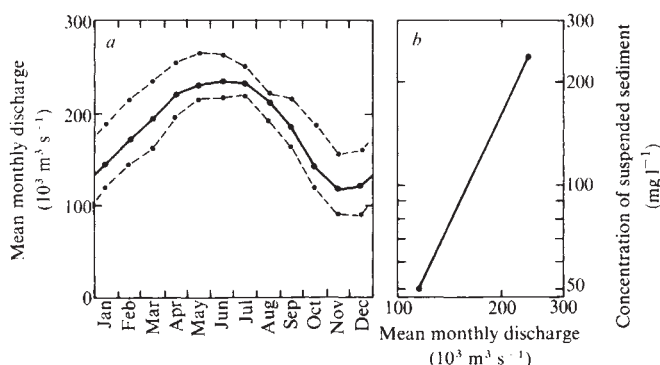


Fig. 3 *a*, Mean monthly discharges of the Amazon River at Óbidos, 1971–75. Maximum and minimum monthly mean discharges are shown by dashed lines; averages are shown by the solid line. Note that the greatest monthly mean flow for the 4-yr period is only 3 times the smallest. *b*, Assumed relation between mean monthly concentration of suspended sediment and mean monthly river discharge at Óbidos.

Photolysis of highly chlorinated dibenzo-*p*-dioxins by sunlight

CHLORINATED dibenzo-*p*-dioxins (CDDs) are now widely recognised as contaminants of commercial chlorinated phenols and related products. These compounds possess a remarkable range of toxicity; the LD₅₀ for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in female rats is 45 μg per kg, that of the octachloro compound (OCDD) is $> 1 \text{ g}$ per kg (ref. 1). Although the degree of toxicity is related to the extent of chlorination, positional isomerisation also plays a critical and perhaps dominant part; 1,2,3,4-TCDD is at least 800 times less toxic than 2,3,7,8-TCDD (ref. 2). The extreme toxicity of some of these compounds, coupled with the large variations with extent of chlorination and positional isomerisation, make accurate

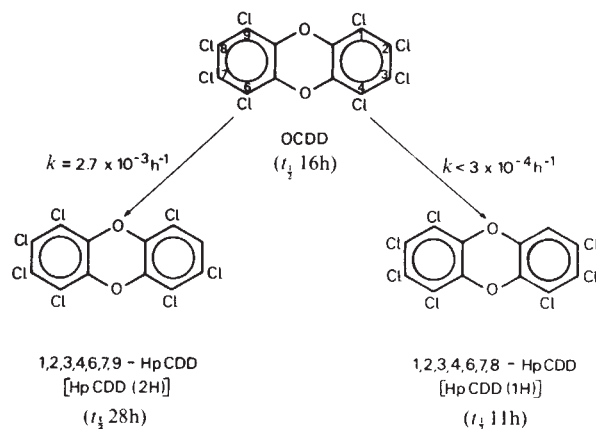


Fig. 1 Photolysis of OCDD in hexane. The rate constants and half lives quoted here derive from an experiment involving consecutive daily exposures of samples in hexane solution contained in matched quartz photolysis cells. The samples were exposed from 09.00 to 15.00 in late September/early October 1977 at Princes Risborough. The kinetics were all found to be first-order when the total time exposed was used (minimum correlation coefficient 0.986 for 10 data points). Hours of direct sunlight did not produce such a good correlation. The experiments have been repeated outside at other times of the year and in the laboratory and once allowance is made for the different incident light intensities the results are in agreement. For example, the half lives of OCDD were found to be 7.0 h at the beginning of August and 9.0 h in mid September. The computed ratios of the rate of OCDD destruction to the rate of conversion of OCDD to HpCDD(2H) are, however, independent of the solar intensity and the same results have been found in laboratory experiments using a fluorescent blacklight for the photolyses. In all cases the concentrations of the solutions used were such that less than 10% of the incident light was absorbed.

identification of CDDs essential in any assessment of environmental or safety implications. The study reported here is concerned with the photolysis of octa-, hepta (Hp)- and some hexa (Hx)-chlorodibenzo-*p*-dioxins and shows that the structural isomers which are expected to be the most toxic are most rapidly photodecomposed. We also report that despite photolytic reductive dechlorination which gives rise to less highly chlorinated dioxins, the reaction mechanism favours the formation of the less toxic isomers.

OCDD is a common contaminant of the widely used biocide pentachlorophenol occurring at a concentration of $\sim 1,000 \mu\text{g}$ per g in the commercial product³. Previous work has shown that photolytic reductive dechlorination can occur^{4,5} and the possible safety implications of this have been considered⁵ but without any detailed consideration of the kinetics or the mechanism of reaction.

Figures 1 and 2 summarise the principal results of our work which, although designed to study the photolysis of OCDD, has also provided data of relevance to other CDDs. The first photolysis experiments, conducted with a solution of OCDD in hexane (approximately $1 \mu\text{g}$ per ml) containing traces of HpCDD isomers, indicated that the OCDD and one of the HpCDD isomers were photolytically decomposed but that the other HpCDD increased in concentration during photolysis. Figure 3 shows gas chromatography (GC) traces illustrating this behaviour. There was also evidence from the GC traces of an increase in concentration of another compound which has a relative retention time on GC in the region expected for a HxCDD⁴. As photolysis continued, and the concentration of OCDD decreased, the concentration of the HpCDD isomer that had previously increased reached a steady state and then decreased. The same behaviour was found when the initial ratios of concentrations of HpCDD and OCDD were changed. The HpCDD is therefore generated by photolysis of OCDD. Photolytically-induced reductive dechlorinations have been reported before for chlorinated dioxins^{4,5} but the identification of the formation of a specific HpCDD facilitates an investigation of the mechanism of reaction.

Highly chlorinated dibenzo-*p*-dioxins can be formed by controlled heating of sodium chlorophenoxides⁶. We prepared OCDD from purified sodium pentachlorophenoxide and confirmed the structure by mass spectroscopy. Other CDD compounds cannot be prepared in the pure state in this way. HpCDD and HxCDD were prepared by the controlled heating of mixtures of sodium pentachlorophenoxide and specific sodium tetrachlorophenoxides^{6,7} and we confirmed their identity by GC analyses on QF-1 and OV-101 columns. The relative retention times with respect to OCDD on OV-101 were in agreement with published values⁸.

Comparison of the GC traces from the photolysed solution on two GC columns with samples of authentic compounds prepared as above showed that the HpCDD which increased in concentration was the 1,2,3,4,6,7,9-HpCDD (or HpCDD(2H)). Similarly, the peak in the HxCDD region could be assigned to the 1,2,4,6,7,9-HxCDD (or HxCDD(2,7H)).

To study the kinetics of photolytic degradation of both the HpCDDs, we minimised the formation of HpCDD from OCDD by varying the ratios of sodium penta- and tetrachlorophenoxides used in the synthesis of CDDs. The ratio of concentrations of HpCDD and OCDD chosen was at least 20 times the concentration ratio at which the photolytic production and destruction of HpCDD(2H) were found to be equal. These solutions also contained HxCDD isomers and the half lives for these compounds in sunlight are shown in Fig. 2.

All the solutions were exposed in matched quartz photolysis cells placed in a rack so that each solution was exposed to the same intensity and spectrum of sunlight. The rate constant for conversion of OCDD to HpCDD(2H) in these conditions (Fig. 1) can be calculated from the ratio of concentrations of these two compounds at the photostationary state for HpCDD(2H). As no ratio of concentrations was found for which the concentration of HpCDD(1H) increased during photolysis, only an upper limit to the rate can be calculated.

It is clear from Fig. 1 that OCDD is short lived when exposed to sunlight in hexane solution. From the rate constants we deduce that reductive dechlorination to produce less chlorinated dioxins is a minor photolytic pathway accounting for $< 10\%$ of the OCDD destroyed. The GC traces showed the photolytic formation of compounds with retention times longer than that for OCDD: these compounds are not simple CDDs and have not been identified.

There are two groups of chlorine atoms in OCDD: those bonded to carbon atoms in the 1-position, which are adjacent to an oxygen substituent (1,4,6 and 9 in Fig. 1) and those bonded to carbon atoms in the 2-position (2,3,7 and 8 in Fig. 1). The half lives (Figs. 1 and 2) indicate that CDDs with chlorine atoms in the 2-position photolyse more rapidly than those with chlorine atoms in the 1-position. This seems to be due partly to the

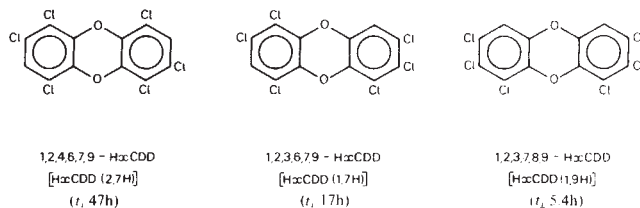


Fig. 2 Photolytic half lives for various hexachlorodibenzo-*p*-dioxin isomers exposed to sunlight in hexane solution in the same conditions as the solutions used for Fig. 1. The solution containing HxCDD(1,9H) also contained some HxCDD(1,6H) which is produced on heating sodium 2,3,4,6-tetrachlorophenoxide^{6,7}. The GC procedure used for the kinetics did not resolve these isomers, initially present in a ratio of $\sim 1:3$, HxCDD(1,6H):HxCDD(1,9H). The decay of the composite peak followed first-order kinetics for a period of over five half lives; as the major component of the peak was the HxCDD(1,9H) the half life found is ascribed to this compound. The half life of the HxCDD(1,6H) must be very similar to the HxCDD(1,9H), otherwise the decay of the composite peak would have been more complex.

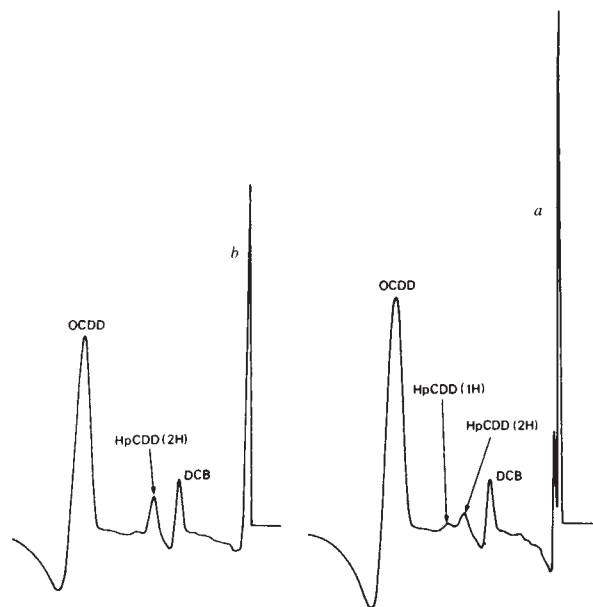


Fig. 3 GC traces from a hexane solution of approximately $4 \mu\text{g ml}^{-1}$ OCDD containing traces of HpCDD impurities before (a) and after (b) exposure of the solution in a quartz photolysis cell to sunlight. The peak labelled DCB is from decachlorobiphenyl ($0.05 \mu\text{g ml}^{-1}$) used as an internal standard for the GC analyses. These chromatograms were run on a Pye 104 gas chromatograph using EC detection. A 1-m column packed with 5% QF-1 on Diatomite C-AW-DCMS 80–100 mesh was used with a column temperature 210°C , detector temperature 300°C and nitrogen gas flow rate 120 ml min^{-1} . Analyses were also carried out with a 1-m column containing 3% OV-101 on Chromosorb W-HP 100–120 mesh, operated with the same temperatures as for the QF-1 column and a nitrogen gas flow rate of $\sim 50 \text{ ml min}^{-1}$. The concentration of OCDD in solutions was found by comparison with standard solutions and approximate HpCDD concentrations found by comparison of peak areas of HpCDD peaks with those for OCDD with allowance for the relative ECD responses of the compounds⁸. Decay constants were found by comparison of photolysed samples with diluted solutions of the unphotolysed solution. The relative retention times for the compounds of interest are:

Compound	Relative retention times		Literature values OV-101 (ref. 8)
	QF-1	OV-101	
OCDD	1.00	1.00	1.00
HpCDD(1H)	0.62	0.59	0.58
HpCDD(2H)	0.57	0.53	0.52
HxCDD(1,6H)	0.39	0.34	0.34
HxCDD(1,7H)	0.36	0.31	0.31
HxCDD(1,9H)	0.44	0.36	0.36
HxCDD(2,7H)	0.32	0.28	0.27
Decachlorobiphenyl	0.42	0.51	—

greater reactivity of the 2-position chlorines, shown by the higher rate of formation of HpCDD(2H) than HpCDD(1H). However, other factors are also involved because compounds possessing the same number of chlorine atoms in the 2-positions photolyse more rapidly as chlorine atoms are removed from the 1-positions ($t_{1/2}\{\text{HxCDD}(1,9\text{H})\} < t_{1/2}\{\text{HpCDD}(1\text{H})\}$) and $t_{1/2}\{\text{HxCDD}(1,7\text{H})\} < t_{1/2}\{\text{HpCDD}(2\text{H})\}$). Extrapolation of these results leads to the prediction that 2,3,7,8-TCDD should be the most photolabile of the chlorinated dibenzo-*p*-dioxins.

Available evidence on CDDs^{1,2}, and the similarities between the behaviour of the CDDs and other compounds with similar molecular shapes⁹, suggest that the presence of chlorine atoms in the 2,3,7 and 8 positions is critical for toxic effects (enzyme inhibition studies suggest at least three chlorine atoms¹⁰). The observations we report are therefore reassuring from the environmental point of view as the molecules that are likely to be the most toxic are the most susceptible to photodegradation, and the photodegradation of highly chlorinated dibenzo-*p*-

dioxins is not a facile route for the generation of the highly toxic, less chlorinated compounds.

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The evolution of the cell-cell recognition system

THE invention of sexuality was one of the milestones in the evolution of organisms. Indeed, in both unicellular and multicellular organisms, sexuality, by allowing the exchange of genetic material between cells, has been the major driving force of evolution. On the other hand, sexuality requires surface structures for the specific recognition and mating of the cells of the two gametic types. One of us (A.M.) has recently suggested that the cell-cell recognition system is phylogenetically linked to the appearance of sexuality¹. A phylogenetic connection between sexuality and cell recognition has also been suggested by Bennett *et al.*². Here, we will elaborate on this idea.

We can only speculate on the organisation of the cell membrane in the primitive prokaryotes; it may have been just a lipid bilayer. The major advance in this organisation must have been the insertion of proteins and/or glycoproteins in the bilayer. This step provided the cell membrane with an immense potential for modulating its molecular organisation; and this may have been the basis for the species-specific organisation of the cell membrane and hence of cell recognition. We do not know this happened concurrently with the appearance of the sexuality factors; in any case, it may be assumed that the sexuality factors were responsible for encoding certain proteins which became inserted into the membrane of the cells carrying such factor(s).

Jacob *et al.*³ have suggested that in bacteria, conjugation depends on an antigen, the F-antigen, at the bacterial surface of the F⁺ of *Hfr* males, which 'would allow the host to recognise cells which do not carry the episome and to conjugate with such cells'. Hence, originally the mating reaction would have resulted from the interaction between surface components specific for the (+) cells (which in *Escherichia coli* seem to be located at the tip of the F-pilus⁴) with some surface component of the (–) cells. Indeed, one might visualise a situation in which the molecules encoded by the sex factor(s) at the surface of the (+) cells were similar to lectins or multivalent antibodies and interacted with some of the glycoprotein components of the surface of the (–) cells in a process similar to 'anchorage modulation' or cap

formation⁵. The suggestion of Jacob *et al.* is supported by recent work of Kennedy *et al.*⁶ which shows that the proteins coded for by the *tra* operon (the transcriptional unit containing the genes required for the synthesis of the F-pili and the stabilisation of the mating aggregates, for DNA transfer and for surface exclusion which prevents the interaction of F cells with other donors) are associated with the cell envelope. The fact that the *tra* operon also codes for a membrane protein (PTraT, which is responsible for surface exclusion)⁷ is particularly important in connection with the origin of the specificity of cell-cell recognition and interaction. It is worth mentioning at this point that the incompatibility system in higher plants also depends on proteins at the surface of the pollen tube whose synthesis is controlled by the S locus (see ref. 8 for review).

Apparently, very early in the course of evolution not only did the molecular organisation of the cell surface fall under genetic control, but also the surface structures involved in conjugation were, in turn, able to control the activity of the genetic apparatus. The observations of Skurray and Reeves⁹ are an example of this. These authors found that in the F⁻ cells certain functions, notably DNA synthesis, are inhibited during conjugation. On the other hand, the location on the bacterial membrane of the initiator site of DNA replication³ is well known. The next step in the evolution of recognition in the mating reaction in lower eukaryotes must have been the establishment of specificity of the 'molecular fit' of the receptor sites. For example, in the yeast *Hansenula wingei* it has been shown that mating results from the interaction of two glycoproteins at the surface of the cells of the two mating types¹⁰⁻¹². In *Clamydomonas*¹³, the putative area of fusion between mt⁺ and mt⁻ cells seems to be a structurally specialised area of the cell surface which differentiates during gamete maturation. However, precise knowledge of the molecular composition and organisation of the receptor sites is still lacking.

We suggest that the cell-cell communication system has evolved from the membrane structures originally meant for cell recognition in the mating process. In this connection, we can cite the experiments of Miyake (see his review, ref. 14) on the ciliate *Blepharisma*. In this organism, the preconjunction events of cell interaction between mating types I and II are mediated by two 'gamones' (gamones 1 and 2) secreted by the cells. Both gamones are able to induce cell union events between cells of the same mating type. In this case, however, the union remains sterile, that is, none of the nuclear events—meiosis, exchange and fusion of gametic nuclei—that occur when cells of the two different mating types unite, will follow. By taking advantage of a mutant, *doublet*, which is provided with two sets of oral openings (cell union occurs in the oral region), chains of cells of the same mating type could be constructed by submitting them to the action of gamone 1. If at this point one singlet (that is, of the wild type with one oral opening) cell of the complementary mating type (pretreated with gamone 2) is attached to one end of such a chain, the conjugation events are immediately activated in a chain-like sequences, that is, they start on the cell closest to the 'inducer' cell and propagate from cell to cell.

Let us now leap from the unicellular to the multicellular organisms. We suggest that this event in evolution was concurrent with the segregation of the somatic from the germ cell line. The dichotomy between the two cell lines involves (1) in the somatic cell line, the silencing of the genetic apparatus coding for the surface structures responsible for the recognition between male and female individuals; (2) in the germ line, the retention of a fully (or at least largely) derepressed genome.

According to this hypothesis the cells of the germ cell line have retained their ancestral surface organisation whereby only cells belonging to the opposite sex can interact with one another. In this connection it may be significant that in vertebrates the primordial germ cells originate far away from the gonadal ridge and migrate as single cells.

On the other hand, although the matter has not been investigated with this question in mind, the formation of mouse chimaeras¹⁵⁻¹⁷ shows that genetically male and female embry-

onic cells do not discriminate between one another. Also, hybrid histotypic aggregates can be formed in culture from such species as far apart as chick and mouse¹⁸⁻²⁰. (However, we must remember that *in vitro* conditions may alter the organisation of the cell surface in such a way that some properties such as the species specificity are lost, while the tissue specificity is retained.) These experiments suggest that the structures discriminating between male and female are not expressed at the surface of these cells.

The retention of a largely derepressed genome by the cells of the germ line is inferred from the fact that in the oocyte, the complexity of the transcripts is several times greater than in the somatic cells (see, for example, ref. 21). Although we know of no such direct evidence in the case of the male germ cells, it has been shown that at least in *Drosophila*, spermatocytes exhibit lampbrush chromosomes comparable to those of the oocyte²².

It is therefore not surprising to find at the surface of the germ cells—both eggs and spermatozoa—antigens and other molecules which are also present at the surface of highly specialised somatic cells. One such example is the presence of acetylcholine receptors at the surface of the *Xenopus*²³ and of the mouse oocyte²⁴. The role of these receptors is obscure; in the mouse oocyte they are no longer detectable in the two-cell embryo²⁴.

Finally, we should mention the relevance here of some of the information now available on the T locus in the mouse. Indeed, it has been shown that mutations at this locus result in both segregation distortions in males and fertility alterations²⁵ and that they also affect cell interactions during the early stages of embryonic development. The antigens encoded by the T locus are expressed at the surface of the spermatozoon but not of any somatic cell, thus suggesting that they may have an important role in gamete differentiation and during early embryonic development²⁶. The location on the same chromosome (and in very close proximity to each other) of the T locus and the H-2 locus which specifies the major histocompatibility antigens may also suggest an evolutionary relationship between the genetic system controlling gamete differentiation, cell recognition and the immune system of the higher vertebrates.

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Microtubules, cell wall deposition and the determination of plant cell shape

THE shape of plant cells depends largely on the orientation of cellulose microfibrils in their cell walls¹. In almost all cells where the microfibrils are regularly arranged, cortical microtubules lie parallel to the wall microfibrils. Experimental evidence indicates that these microtubules influence the arrangement of the deposition of wall microfibrils². How microtubules determine the orientation of microfibrillar deposition is not yet known^{3,4}, but it has been speculated that they may influence the alignment of cellulose synthetase enzymes on the plasma membrane⁵. Microtubules act as a cytoskeleton in the formation and maintenance of cell shape in many types of cells⁶ including plant cells which lack a cell wall⁷, or before a wall is deposited⁸, and it may be that microtubules, in those plant cells where they are arranged congruently with recently deposited wall microfibrils, directly determine cell shape. However, using coumarin to inhibit cell wall regeneration by protoplasts of *Mougeotia* I demonstrate here the inability of microtubules to directly determine cell shape.

Spherical protoplasts of this freshwater alga, isolated following enzymatic digestion of its cell wall, readily regenerate a new wall and revert to their original cylindrical cell shape⁹. The ratio of length to diameter (axial ratio) of these cells is very large, making them ideal for investigations on the mechanisms controlling the development of cell asymmetry. Following attachment to a substratum, these protoplasts can be burst by osmotic shock to reveal the cytoplasmic face of the plasma membrane and microtubules associated with it¹⁰. Cortical microtubules become linked by cross bridges to the membrane during the first 3 h of protoplast culture (unpublished data). Intact protoplasts treated with 0.2% colchicine or 10⁻⁴ M isopropyl *n*-phenylcarbamate (IPC) lose their cortical microtubules¹⁰ and regenerate a cell wall in which the microfibrils are randomly orientated. These cells remain spherical (unpublished data).

Coumarin (1,2-benzopyrone) inhibits the biosynthesis of cell wall components, especially cellulose¹¹, and prevents the deposition of microfibrils on the surface of regenerating tobacco

leaf protoplasts¹². This drug does not affect the cortical microtubules of plant cells¹³. Freshly isolated protoplasts of *Mougeotia* were washed in protoplast maintenance medium (PMM), which contains 0.2 M mannitol, 0.2 M sorbitol, 2 mM CaCl₂·2H₂O and 2 mM NaH₂PO₄ at pH 5.5. A stock solution of 50 mg ml⁻¹ coumarin (Sigma) in ethanol was diluted with PMM to 250 µg ml⁻¹. Protoplasts were incubated in this solution at 20 °C on the same 16/8 h light/dark regime they had experienced before removal of their cell wall. Control protoplasts were maintained in PMM containing 0.5% ethanol. Wall regeneration by the protoplasts was monitored by staining with 0.1% Calcofluor M2R New (American Cyanamid), a fluorescent stain for β -polysaccharides¹⁴, as well as by transmission electron microscopy.

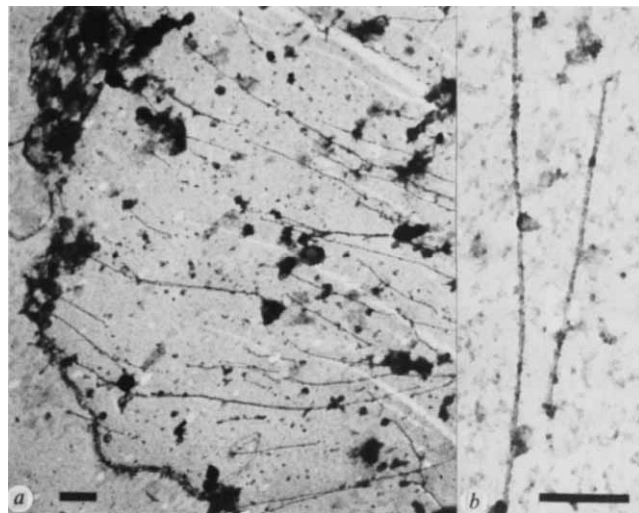


Fig. 2 *a*, Transmission electron micrograph of part of a *Mougeotia* protoplast plasma membrane. Protoplasts were allowed to settle on formvar/carbon-coated grids treated with poly-L-lysine. They were then burst by osmotic shock in a microtubule stabilising buffer containing 1 mM MgSO₄, 2 mM EGTA and 100 mM PIPES at pH 6.9 and negatively stained with uranyl acetate. This protoplast had been incubated in 250 µg ml⁻¹ coumarin for 24 h before bursting. Microtubules are conspicuous on the membrane. Scale marker = 1 µm. *b*, High magnification micrograph of the plasma membrane of a burst coumarin-treated protoplast showing microtubules. Scale marker = 0.5 µm.

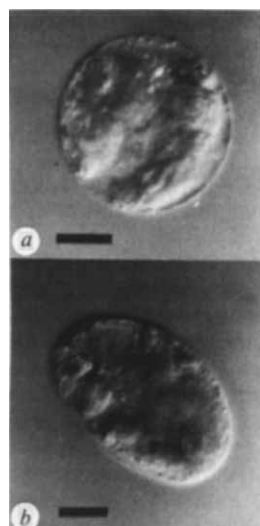


Fig. 1 *a*, Photomicrograph of a protoplast of *Mougeotia* that has been cultured in 250 µg ml⁻¹ coumarin in PMM for 24 h; it lacks a cell wall and remains spherical. Scale marker = 10 µm. *b*, Normal regeneration of a *Mougeotia* protoplast after 24 h in PMM without coumarin. The cell has a wall and is ovoid in shape. Scale marker = 10 µm.

Over 50% of untreated protoplasts regenerate a cell wall after 5 h in culture, and by 24 h over 90% have a wall. Such regenerating cells become ovoid after about 12 h, cylindrical within 2 d, and by the third day of culture many have divided⁹. Protoplasts treated with coumarin for up to 3 d do not develop a cell wall that is detectable by electron microscopy or by Calcofluor fluorescence, and they remain spherical (Fig. 1). These protoplasts could be ruptured by osmotic shock (further evidence for their lack of a cell wall) and contain microtubules attached to the plasma membrane (Fig. 2). The cortical microtubular arrays are indistinguishable from those in untreated cells. If coumarin-treated protoplasts were transferred to PMM lacking coumarin they develop a cell wall and become cylindrical; the time course of this belated development is similar to that of the controls.

If coumarin does not affect microtubules, as suggested by this work and that of Itoh¹³, they apparently have no direct cytoskeletal function in developing the shape of these cells as they do in *Ochromonas*⁷, *Pediastrum*¹⁵ and some higher plants¹⁶. The finding that coumarin-treated protoplasts do not deposit a cell wall and that they remain spherical clearly implies that the wall is necessary for establishing cell shape as well as for its maintenance. Experiments with colchicine and IPC suggest that, as in

higher plants, cortical microtubules control cell shape by influencing the pattern of microfibril deposition.

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example, from 1 to 3%, without affecting the separation significantly. Addition of anti-clumping agents such as 12 mM xylocaine or 0.01% EDTA⁹, although reducing clumping to a minimum, also reduced cell viability by up to 40%. Substitution of phosphate-buffered saline (PBS) for culture medium led to slightly increased clumping, and the presence of fetal calf serum in either the culture medium or PBS caused marked clumping of the fibroblasts.

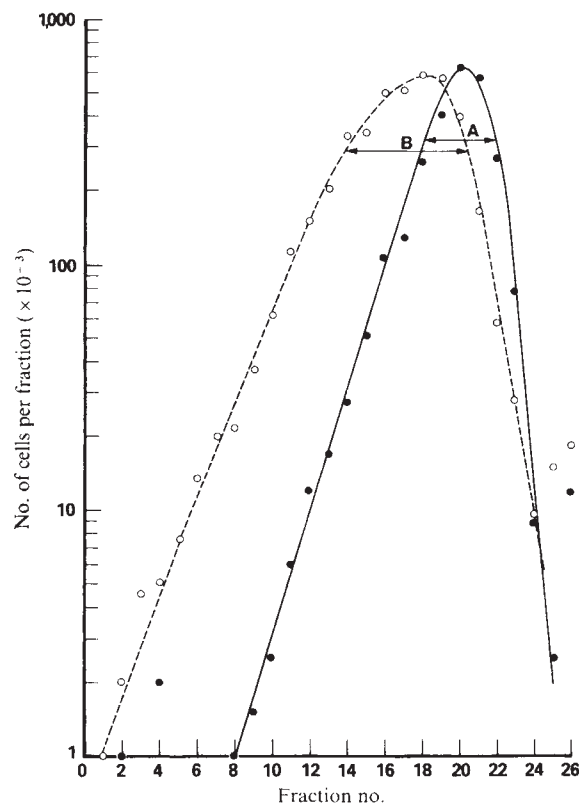


Fig. 1 Sedimentation profiles of unsynchronised fibroblasts and fibroblasts synchronised by serum starvation. Approximately 5×10^6 human skin fibroblasts cultured in Ham's F-10 medium supplemented with 20% fetal calf serum, glutamine and antibiotics, or a similar number of fibroblasts synchronised by serum starvation (0.2% of fetal calf serum in Ham's F-10 medium for 3 d) were collected with trypsin (0.125%) and EDTA (0.02%). Cells were suspended in 20 ml of Ham's F-10 medium and layered as a thin band on top of a gradient of 2-3% bovine serum albumin in Ham's F-10 medium in the staput apparatus with a 10-ml pipette. After sedimentation at 4 °C for 3 h, 50-ml fractions were collected from the bottom of the gradient. Each fraction was centrifuged at 200g for 10 min, and the pellets were suspended in 0.5 ml of complete medium for cell counts. ○, Unsynchronised fibroblasts; ●, fibroblasts synchronised by serum starvation.

A further complication with cultured fibroblasts was the variability in size among the diploid parental cells, in part due to their being in different phases of the cell cycle. To bring them to a homogeneous size range before fusion and thus maximise the size difference between the fused and unfused cells after fusion, techniques known to induce cell synchrony were tried. We found that serum starvation reduced considerably the variation in cell size (Fig. 1), so that the width of distribution at half peak height was 2.4 units (B) for the serum-starved population compared to 4.1 units (A) for the untreated cells. Thus by this treatment fewer larger cells and a predominance of smaller cells were obtained. Synchronisation by leaving cells to grow to confluence, or by single or double thymidine block was less successful than serum starvation in reducing the variation of cell size, and furthermore, thymidine treatment tended to increase clumping.

Non-selective isolation of human somatic cell hybrids by unit-gravity sedimentation

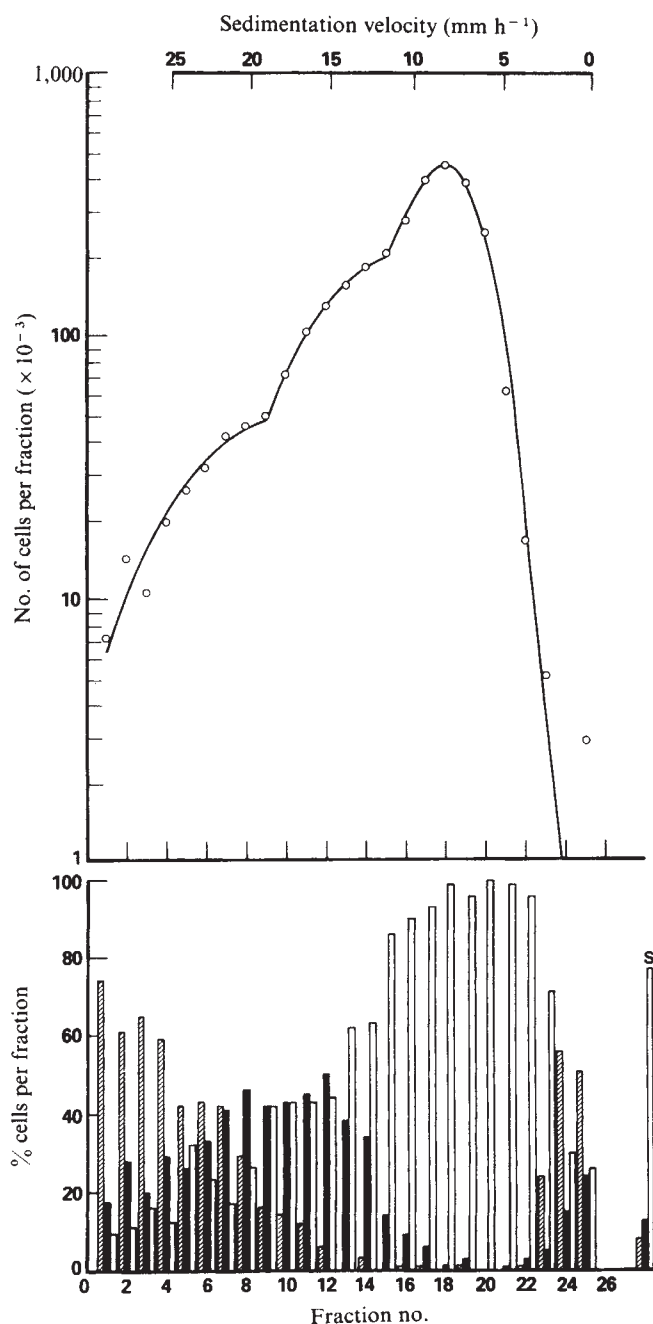
DATA from somatic cell hybridisation have made important contributions to the understanding of genetic control of phenotypic expression, the nature of malignancy in tumours, gene mapping and the basic genetic lesions causing metabolic diseases¹⁻⁵. Broader application has been limited by the absence of biochemical markers in most cell strains and lines, making it difficult to eliminate parental cells and isolate hybrids. Recent non-selective methods of isolating hybrid cells require either expensive and elaborate equipment⁶ or examination of large numbers of clones with a low yield of hybrids⁷. We now present a method for isolating tetraploid clones from fused human fibroblasts non-selectively and in high yield. Our technique is applicable to tetraploid cells formed from any pair of diploid cells as long as the synkaryons so formed can proliferate. The method depends on the separation of fused cells from the unfused parental cells because of their greater size. A mixture of cells settles out according to size in a shallow bovine serum albumin (BSA) gradient at unit gravity, yielding fractions enriched in double-nucleated cells from which tetraploid clones can be isolated in high yield.

Our goal was to fuse human fibroblasts with polyethylene glycol and then fractionate them by means of a 'staput' chamber⁸. A thin layer of fused cell suspension was pipetted on top of a shallow gradient solution in the chamber and left to settle at unit gravity. In these conditions, sedimentation velocity was almost directly proportional to the square of the cell radius⁸, so that the larger fused cells settled out faster than the smaller unfused cells. After the specific time for differential sedimentation had elapsed, cell fractions were collected from the bottom of the gradient.

To keep the fibroblasts in a monodispersed state for effective separation and to maintain their maximal viability for cloning, we found that BSA dissolved in otherwise serum-free culture medium (Ham's F-10) was superior to Ficoll or fetal calf serum as the gradient material. Because the purpose of the gradient was to prevent adjacent layers from mixing during collection, the percentage of BSA in the gradient could be varied, for

After synchronising human skin fibroblast strains by serum starvation, we fused two strains with polyethylene glycol 1000 (ref. 10) and fractionated the cells according to size in the staput chamber containing a gradient of 2–3% BSA dissolved in serum-free Ham's F-10 culture medium (Fig. 2). Because cells with more than two nuclei do not divide¹¹, they could be ignored for subsequent cloning purposes. However, fractions that contained the highest ratio of double-nucleated cells to single-nucleated cells should have the best chance of yielding tetraploid clones. Therefore, the earliest fractions (1–8) collected from the bottom of the sedimentation chamber with an average ratio of double-nucleated to single-nucleated cells of 2:1 were used for cloning. In contrast, the original unsorted sample had an average ratio of 0.17:1.

Of the 72 clones successfully propagated for identification by karyotyping¹², 18 (25%) had a modal number of 46 (diploid), nine (12.5%) had both diploid and tetraploid cells and 45 (62.5%) were tetraploid. Several tetraploid clones were carried through multiple passages, each maintaining their complement of 92 chromosomes.



Vigorous growth capability in both parent cell strains was essential for successful propagation of tetraploid clones. For example, when we fused a female skin fibroblast strain with a male skin fibroblast strain that was much more vigorous in growth, we found that of the 45 tetraploid clones, at least 24 were apparently from male-male fusions with two Y chromosomes. Only nine clones were judged to be from female-female fusions, as shown by the absence of a Y chromosome, and 10 were judged to be from male-female fusions, as shown by the presence of only one Y chromosome. The identification of the last two categories was tentative, as the complete tetraploid chromosome number of 92 was seldom obtained. The number of chromosomes counted was most often approximately 80, mainly because of the technical difficulty in spreading so many chromosomes properly for counting and identification.

To overcome this difficulty of identifying true hybrids cytogenetically, we used the isozymes of glucose-6-phosphate dehydrogenase (G6PD) as markers^{13,14}. A fibroblast strain with the G6PD_A isozyme was fused with another strain with the G6PD_B form. After separation in the staput chamber, clones propagated from fractions 1–8 were identified cytogenetically as diploid or tetraploid. All the tetraploid clones were then subjected to electrophoresis to identify their G6PD profile. Tetraploids formed from homologous fusion between the same parent cell types would show either the A or the B form. Hybrid tetraploids formed from heterologous fusion between the two parent cell types would show the hybrid heteropolymer between the A and B form in the ratio of 1:2:1. Out of 202 clones isolated from fractions 1–8, 114 were growing sufficiently vigorously for karyotyping. Of these, 31 were tetraploid, seven were mixed tetraploid and diploid and 76 were diploid. Electrophoretic separation of G6PD isozymes in 30 of the tetraploid clones showed that six had the typical hybrid pattern, 21 had only the A form, one had the appearance of the hybrid form mixed with the A form, 1 had the B form and one had both A and B. Thus, of the 30 clones judged to be tetraploids, 20% were found to be pure hybrid clones.

We have thus shown that by exploiting the different sizes of hybrid cells and their unfused parents, hybrid tetraploid clones can be obtained in high yield. The staput apparatus provides a relatively simple technique for separating somatic cell hybrids and is applicable to a wide variety of cell types which have not been studied previously because of their lack of biochemical selection markers. Furthermore, because hybrid clones can be isolated without perturbing the genetic composition of the parental cell strains by chemical or physical selection, the exact biochemical and genetic mechanisms that account for complementation between some types of inborn error of metabolism may be more easily revealed and questions of gene dosage and gene inactivation can be examined in the hybrid clones without constraint.

Financial support from the MRC, Canada (MA-5789), technical assistance from M. Gedeon, A. Davidson and Dr N. Rosa and advice in karyotyping from Dr I. Uchida and her staff are gratefully acknowledged. The G6PD_A cell strain was provided

Fig. 2 Separation of human skin fibroblasts by unit-gravity sedimentation in the staput apparatus after fusion with polyethylene glycol. Fibroblasts synchronised by serum starvation were seeded in T-75 culture flasks (Corning) at a density of 4×10^6 cells per flask (2×10^6 cells from each of two fibroblast cell strains) and incubated for 24 h in complete medium (Ham's F-10 supplemented with 20% fetal calf serum). They were fused with polyethylene glycol 1000 according to the procedure of Davidson *et al.*¹⁰ and incubated for about 16 h before collection for separation in the staput apparatus, as described in the legend to Fig. 1. After sedimentation at 4 °C for 4 h, 50-ml fractions were collected from the bottom and centrifuged at 200g for 10 min. The pellet was suspended in 0.5 ml of complete medium for cell count, Giemsa staining and cloning. The entire operation was performed in sterile conditions. —○—, No. of cells per fraction; S, sample cell load before sedimentation. Open columns, one nucleus per cell; solid columns, two nuclei per cell; hatched columns, more than two nuclei per cell.

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Infection of the Mongolian gerbil with the cattle piroplasm *Babesia divergens*

DISEASES of cattle caused by piroplasms are one of the major constraints on the growth of the livestock industry in the tropics. *Babesia divergens*, which is the common cause of redwater in European cattle, can be transmitted to splenectomised goats, sheep and deer by the inoculation of blood from infected cattle¹. Furthermore, it has been shown experimentally that *B. divergens* is transmissible in this way to chimpanzees². Evidence is now accumulating to suggest that it is this species of *Babesia* which is capable of causing fatalities in splenectomised humans in Europe^{3,4}. Our inability to adapt cattle piroplasms to small laboratory animals has restricted efforts to develop new drugs

Table 1 *B. divergens* parasitaemias of infected gerbils up to 96 h post-inoculation

Time post-inoculation (h)	No. of infected erythrocytes per 1,000			
	G1	G2	G3	G4
24	<1	<1	<1	<1
48	<1	<1	<1	<1
72	58	17	7	7
96	372	50	236	67

G1 and G2 were splenectomised gerbils; G3 and G4 were intact gerbils.

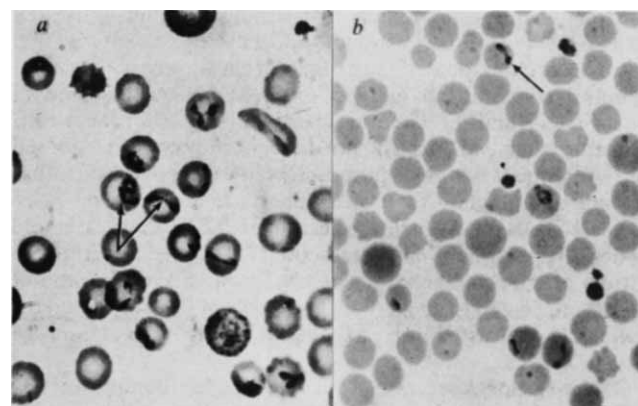


Fig. 1 *B. divergens* piroplasms infecting *a*, gerbil, and *b*, bovine erythrocytes. Giemsa-stain, $\times 2,200$. Arrows indicate characteristic divergent forms of the parasite.

and vaccines against them. However, during a recent investigation of a human fatality⁵, we were able to transmit the parasite to gerbils by inoculation of infected blood from the patient. Although final evidence to implicate *B. divergens* in this instance is still being assessed, we decided to investigate the possibility of infecting gerbils with *B. divergens*-infected blood from cattle. We now report the successful adaptation of *B. divergens* to the Mongolian gerbil (*Meriones unguiculatus*).

A splenectomised Friesian calf reacting to inoculation with a strain of *B. divergens* originally isolated from Eire had a *B. divergens* parasitaemia of 61 infected erythrocytes per 1,000 cells. A sample of 75 ml of blood was taken from the calf's jugular vein into Anticlot (Clinton Laboratories) and centrifuged at 3,400g for 20 min. The resultant pellet was resuspended and washed three times in phosphate-buffered saline (PBS) (pH 7.2). The pellet was finally reconstituted so that 0.7 ml contained $\sim 5 \times 10^8$ *B. divergens*-infected erythrocytes.

Four gerbils, two intact and two splenectomised, were each inoculated intraperitoneally with 0.7 ml of the reconstituted blood. Thin blood smears were made daily from the gerbils' tail-tips and stained with Giemsa. Parasites were seen in blood smears from all animals from day 1 post-inoculation and parasitaemias are shown in Table 1. Parasites present in the blood of infected gerbils exhibited considerable pleomorphism (Fig. 1*a*); however, several of these forms were characteristic of *B. divergens* piroplasms in infected bovine erythrocytes (see Fig. 1*b*).

On day 4 post-infection all the gerbils had significant *B. divergens* parasitaemias. One intact animal with a 23% infection of *B. divergens* piroplasms was killed and 0.1 ml of blood containing 2×10^7 infected erythrocytes was inoculated intraperitoneally into two further gerbils (G5 and G6). On the fourth day after inoculation gerbil G5 had a parasitaemia of 14 infected erythrocytes per 1,000 and a red blood cell (RBC) count of 8.9×10^9 per ml; gerbil G6 had a parasitaemia of 59 per 1,000 erythrocytes and an RBC of 6.2×10^9 per ml. They were killed and ~ 1.5 ml of blood was recovered from each animal, representing 1.8×10^8 and 5.5×10^8 *B. divergens*-infected erythrocytes, respectively. The erythrocytes were washed and

Table 2 Summarised reactions of splenectomised Friesian calves to the inoculation of approximately 10^8 *B. divergens*-infected erythrocytes

Calf no.	No. of infected erythrocytes inoculated	Origin of parasites inoculated	Parasitaemia		Febrile response		Haematology	
			Prepatent period to parasitaemia of 1 per 1,000 RBC (d)	Maximum parasitaemia per 1,000 RBC	Prepatent period to febrile response of $\geq 39.5^\circ\text{C}$ (d)	Maximum febrile response ($^\circ\text{C}$)	Lowest PCV (%)	Highest MCV (μm^3)
M1010	1.8×10^8	Gerbil	4	143	6	40.7	15.1	46
M1011	5.5×10^8	Gerbil	4	180	4	41.2	10.2	47
J488	9.7×10^7	Calf	3	186	3	40.5	8.9	44

PCV, packed corpuscular volume; MCV, mean corpuscular volume.

resuspended in PBS as before. Splenectomised calf M1010 was then inoculated intravenously with parasites from gerbil G5 and splenectomised calf M1011 with parasites from gerbil G6. A further 0.2 ml of blood from each infected gerbil was inoculated into a splenectomised control gerbil. Both control gerbils subsequently experienced similar reactions to those of the animals used in the first passage of bovine blood.

The reactions of the two splenectomised calves and that of a splenectomised control calf inoculated with 9.7×10^7 *B. divergens* parasites (Eire strain) are summarised in Table 2.

We have thus demonstrated (1) that gerbils can be infected with *B. divergens* by the intraperitoneal inoculation of infected bovine blood; (2) that the parasite is then transmissible to further gerbils; (3) that the parasite can be used to infect splenectomised calves once again after two gerbil passages.

The epizootiological significance of an alternative rodent host for piroplasms affecting cattle merits further investigation. Furthermore, these preliminary findings seem to indicate that the Mongolian gerbil could provide a small animal model for *B. divergens* and other species of cattle piroplasms. This system may prove suitable for immunological investigations and the screening of anti-babesial drugs.

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T-cell lines which cooperate in generation of specific cytolytic activity

THE unidirectional mixed leukocyte culture (MLC) is characterised by cell proliferation and by the development of cytolytic T lymphocytes (CTL)^{1–3}. In murine MLC, the generation of CTL seems to involve proliferation of at least two subpopulations of responding T cells—T cells of the Ly-2⁺3⁺ phenotype, which differentiate into CTL, and cells of the Ly-1⁺ phenotype, which seem to 'help' or 'amplify' the CTL response^{4–6}. The mechanism by which 'amplifier cells' augment the CTL response is unclear; recent reports have shown, however, that CTL precursors present in populations of long-term MLC or of immune spleen cells^{7,8} can respond to supernatant fluid obtained from allogeneic MLC or mitogen-stimulated spleen cell cultures in the absence of specific antigen. Several groups using such conditioned medium have reported the successful long-term culture of normal and antigen-specific immune T cells in the absence of alloantigen stimuli^{9–11}. Whereas amplifier supernatant fluids seem to substitute functionally for a T-cell subpopulation (Ly-1⁺?) in the absence of alloantigen, there is only indirect evidence that amplifier cells can be activated by alloantigen to release one or several soluble factors that influence the differentiation or proliferation of

Table 1 Peak levels of ³H-thymidine incorporation

Responding cell	Factor	Stimulating cell		
		Medium	C57BL/6	DBA/2
L2	Medium	197 ± 52	1,104 ± 58	7,501 ± 1,011
	LCA SF	558 ± 384	10,182 ± 5,354	17,228 ± 4,380
	2° MLC SF	1,097 ± 444	1,646*	8,705 ± 1,883
L3	Medium	107 ± 48	1,030 ± 368	1,296 ± 176
	LCA SF	702 ± 490	30,865 ± 12,043	53,313 ± 13,744
	2° MLC SF	925 ± 585	3,242*	13,035 ± 1,522

The incorporation of ³H-thymidine by L2 or L3 cells cultured with irradiated C57BL/6 (syngeneic) or DBA/2 (allogeneic) spleen cells, or medium alone, was measured in the presence of either LCA or 2° MLC supernatant fluid (SF), or medium alone. Values are mean d.p.m. ± s.e.m. Results from 12 different experiments are included. For details, see Fig. 1.

*Mean d.p.m. calculated from results obtained in two different experiments.

CTL^{12–14}. We have used modifications of techniques previously described for the long-term culture of immune T cells to isolate several cloned lines of MLC-reactive T cells, including a non-cytolytic T-cell line which, when co-cultured with alloantigen, permits another cell line to proliferate and express specific cytolytic activity.

Primary MLC were prepared by culturing, for 14 d, C57BL/6 (H-2^b) responding spleen cells with irradiated DBA/2 (H-2^d) spleen cells¹⁵. Cells were then collected and restimulated at low density with irradiated DBA/2 spleen cells¹⁶. After 48 h of secondary MLC, cells were collected and cultured at limiting dilution with irradiated DBA/2 spleen cells using medium consisting of equal volumes of 48-h secondary MLC supernatant fluid and Dulbecco's modified Eagle's medium (DMEM)¹⁷ containing 20% fetal calf serum (FCS). Clones of cells appeared in 30% of the wells 8 to 10 d after culturing. Screening of all clones by a micro-⁵¹Cr release assay¹⁸ revealed cytolytic activity by some, but not all, of the cells. Several lines of such cells, some of which are cytolytic, have been maintained in culture for 7 months by weekly transfer with irradiated DBA/2 spleen cells and supernatant fluid obtained from either 48-h secondary MLC (2° MLC) or 48-h cultures of Lewis rat spleen cells stimulated with concanavalin A (LCA). Two of these cell lines have been studied intensively. Both are T cells, as determined by treatment with anti-Thy 1.2 sera: one, designated L2, is non-cytolytic; the other, designated L3, is cytolytic.

The proliferative response of L2, L3, or a mixture of L2 and L3 (L2 + L3), indicated by incorporation of ³H-thymidine (³H-TdR), was measured on days 3 and 5 in nine different experiments (Fig. 1a, b). The L2 cells cultured with the original stimulating alloantigen (DBA/2) exhibited peak proliferative responses on day 3, with an average stimulation index of 6.8, when compared with the response of L2 cells cultured with irradiated syngeneic C57BL/6 cells. The proliferative response of L2 cells cultured alone or with irradiated C57BL/6 cells did not change significantly between days 3 and 5, and in terms of viable cells recovered, there was only a 1-to-3-fold increase over L2 cell input compared to a 21- to 44-fold increase in viable cells recovered from cultures of L2 cells and irradiated DBA/2 spleen cells.

In contrast, the proliferative response of L3 cells cultured with irradiated alloantigen was not greater than that observed with L3 cells cultured with syngeneic spleen cells (stimulation index of 1.3 for peak response), and was 5.4 times lower than the response of L2 cells cultured with alloantigen. There was no change in the proliferative response of L3 cells between days 3 and 5; viable cells recovered from cultures of L3 cells with irradiated DBA/2 or C57BL/6 cells increased only 1- to 4-fold over L3 cell input compared to a 0- to 1-fold increase for L3 cells cultured alone.

When L2 and L3 cells were cultured together with irradiated DBA/2 spleen cells, the extent of maximal ³H-TdR incorporation on day 3 appeared to be approximately the sum of the values observed with separate populations of L2 and L3 cells (average stimulation index of 14.2 compared to L2 + L3 cells

cultured with irradiated syngeneic C57BL/6 spleen cells). However, the total cell recovery from cultures of L2 + L3 cells with irradiated DBA/2 cells was, on average, twice the sum of the cell recoveries from separate cultures of L2 and L3 cells.

Cytolytic activity was measured with a ^{51}Cr release assay¹⁵ after 3 and 5 days of culture. Although L2 cells proliferated when stimulated with alloantigen in culture (Fig. 1a), these cells never expressed any detectable cytolytic activity (Fig. 1c). Likewise, L3 cells expressed only barely detectable levels of cytolytic activity (mean of <2 lytic units per culture). However, L2 and L3 cells cultured together with irradiated DBA/2 alloantigen developed high levels of cytolytic activity against P-815 target tumour cells (DBA/2 origin) (mean of 71 lytic units per culture). On the other hand, L2 and L3 cells cultured together with irradiated syngeneic spleen cells developed only little lytic activity (mean <4 lytic units per culture). Thus, the inclusion of L2 cells with irradiated DBA/2 alloantigen resulted in an average 22.4-fold increase in cytolytic activity.

Like other investigators⁹⁻¹¹, we have found that 2^0 MLC or LCA supernatant fluid makes possible the proliferation of T cells and the generation of cytolytic activity. The peak proliferative responses for L2 and L3 cells cultured with various supernatant fluids are shown in Table 1. The incorporation of ^3H -TdR by L2 cells cultured with irradiated allogeneic (DBA/2) or syngeneic C57BL/6 spleen cells was significantly higher, on the average, if LCA supernatant fluid was added, as was the total cell recovery. However, the addition of 2^0 MLC supernatant fluid to L2 cells cultured with irradiated DBA/2 cells did not result in any significant increase in either ^3H -TdR incorporation or total cell recovery, except that, in a single experiment, the addition of 2^0 MLC supernatant fluid to L2 cells cultured with irradiated C57BL/6 cells increased total cell recovery 10-fold without any significant increase in ^3H -TdR incorporation. Thus, in the absence of alloantigen, L2 cells cultured with irradiated syngeneic cells could be stimulated to proliferate by the addition of either 2^0 MLC or LCA supernatant fluid. Cytolytic activity, however, was not detectable under any conditions in which L2 cells proliferated.

The effect of 2^0 MLC or LCA supernatant fluid on the peak proliferative response of L3 cells was much greater than the effect on L2 cells (Table 1). The addition of LCA supernatant

fluid to L3 cells cultured with irradiated syngeneic or allogeneic spleen cells increased both the average ^3H -TdR incorporation and cell recoveries 30- to 41-fold, compared to the effect of culture medium only. The effect of 2^0 MLC supernatant fluid was much less than that of LCA supernatant fluid. As seen in Table 1, neither L2 nor L3 cells gave an appreciable proliferative response when they were cultured with 2^0 MLC or LCA supernatant fluid in the absence of additional irradiated cells.

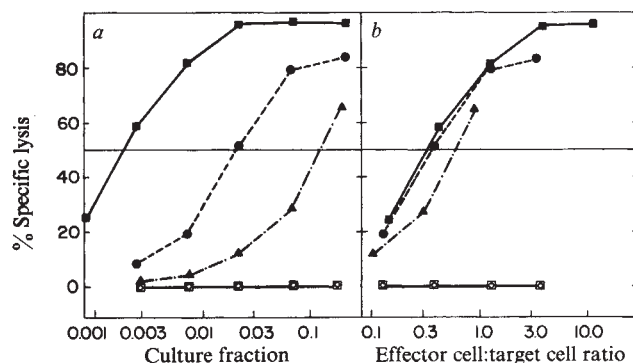


Fig. 2 Cytolytic activity generated by L3 cells cultured with irradiated syngeneic C57BL/6 spleen cells in the presence of LCA supernatant fluid (□), 2^0 MLC supernatant fluid (○), or medium alone (△) after 5 d of culture. Closed symbols, P-815 target cells. Open symbols, AKR-A target cells. a, Cytolytic activity expressed in terms of per cent specific lysis per culture fraction. b, Cytolytic activity expressed in terms of per cent specific lysis at various ratios of effector cells to target cells. For details, see Fig. 1.

These results differ from those reported by Gillis and Smith¹⁰, who found that such supernatant fluids alone were capable of stimulating the proliferation of T cells without addition of cells.

In marked contrast to the results observed with L2 cells, cytolytic activity was detectable whenever L3 cells were stimulated to proliferate. There was a good correlation between the magnitude of the proliferative response and the extent of CTL generation. Figure 2 shows the results obtained in a single representative experiment in which cytolytic activity was

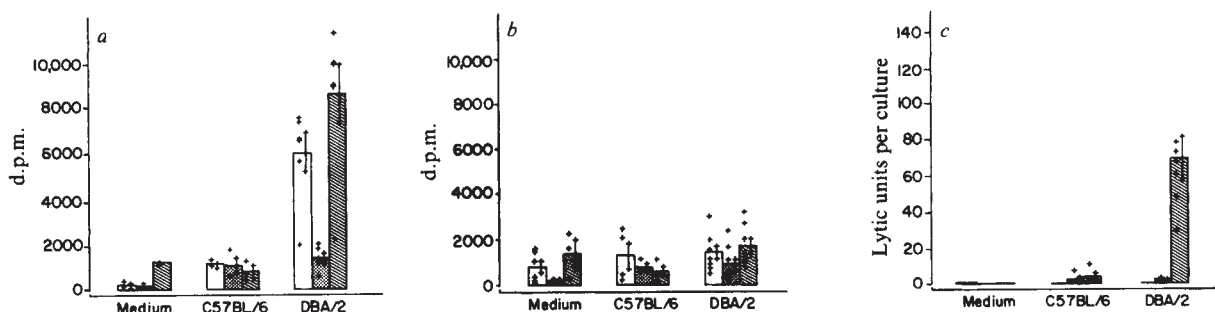


Fig. 1 a, Tritiated-thymidine (^3H -TdR) incorporation by L2 cells (□), L3 cells (▤), and a mixture of L2 and L3 cells (▨) when the cells were cultured alone or with irradiated syngeneic (C57BL/6) or allogeneic (DBA/2) spleen cells in culture medium for 3 d. b, ^3H -TdR incorporation measured after 5 d of culture. c, Cytolytic activity measured against P-815 target cells after 5 d of culture. Results from nine different experiments are presented in which, typically, 1.6×10^4 L2 and/or L3 cells (7 to 8 d after previous transfer) in 0.1 ml were transferred to 3 ml culture wells (Linbro 24-well plate, type 76-033-05) containing 6×10^6 irradiated (1,500 rad) syngeneic (C57BL/6) or allogeneic (DBA/2) spleen cells, or culture medium (DMEM supplemented with 2% heat-inactivated fetal calf serum, 10 mM MOPS buffer, $5 \times 10^{-5}\text{M}$ 2-mercaptoethanol, plus penicillin/streptomycin¹⁷), and either 48-h 2^0 MLC or LCA supernatant fluid (final concentration, 33%), or culture medium, in a final volume of 1.5 ml. Culture plates were then incubated in a humidified atmosphere of 5% CO_2 at 37°C . After 3 and 5 d of culture, individual wells were collected and assayed for both ^3H -TdR incorporation and cytolytic activity. A 0.1-ml culture fraction of each well was placed in triplicate wells of a flat-bottomed microwell tissue culture plate and pulsed with $1 \mu\text{Ci}$ ^3H -TdR (0.36 Ci mmol⁻¹) for 4 h at 37°C . Cells were then collected on to filter paper strips with an Otto Hiller cell harvester and counted for 2 min in a Searle liquid scintillation counter. Results are expressed as d.p.m. $\pm$ s.e.m. Cytolytic activity was determined with a ^{51}Cr release assay¹⁵. Tumour target cells were labelled with $\text{Na}_2^{51}\text{CrO}_4$ and mixed with serial threefold dilutions of effector cells (replicate wells) in round-bottomed microtitre plates. The plates were incubated for 3.5 h at 37°C and then centrifuged, after which one-half of the supernatant volume in each well was counted in a Packard well-type scintillation counter. The per cent of specific cytotoxicity at various effector-to-target cell ratios was calculated by the formula

$$\% \text{ specific lysis} = \frac{\text{c.p.m. exp.} - \text{c.p.m. spontaneous release}}{\text{c.p.m. max. release} - \text{c.p.m. spontaneous release}} \times 100$$

Maximum release was determined by freeze-thaw lysis of target cells in water; spontaneous release was determined from the amount of isotope released during the assay by target cells incubated with medium only. One lytic unit is defined as the number of effector cells required to lyse 50% of 10,000 target cells within 3.5 h.

compared in terms of culture fraction as well as effector-to-target cell ratio for L3 cells cultured with irradiated C57BL/6 spleen cells in the presence of medium, 2^0 MLC, or LCA supernatant fluid. Measured in lytic units (LU) per culture (Fig. 2a), the addition of LCA supernatant resulted in the expression of the greatest cytolytic activity observed (526 LU per culture) which was approximately 11 times greater than that observed with 2^0 MLC supernatant fluid (46 LU per culture) and 67 times greater than that observed with medium alone. Cytolytic activity was directed specifically towards the original stimulating alloantigen, since there was no detectable lysis when syngeneic or third-party allogeneic tumour cells were used (EL-4, AKR-A, respectively). These differences in CTL activity correlated very well with ^3H -TdR incorporation as well as cell recovery.

When evaluated on the basis of effector-to-target cell ratio, the cytolytic activity generated by L3 cells in response to the addition of 2^0 MLC or LCA supernatant fluid, or of medium alone in the presence of irradiated syngeneic cells, was approximately equal (Fig. 2b). Thus, 50% lysis of 10,000 target cells was observed at effector-to-target cell ratios of 0.36, 0.31 and 0.58, respectively. When L3 cells are cultured with irradiated cells and either medium, 2^0 MLC, or LCA supernatant fluids, they appear to have similar killing potential. Thus, the cytolytic activity of L3 cells is related primarily to the number of cells developing during culture; the 2^0 MLC or LCA supernatant fluids seem to stimulate the proliferation of T cells.

These experiments can be summarised as follows: two cloned lines of T cells, L2 and L3, have been obtained from MLC prepared by co-culturing responding C57BL/6 spleen cells with irradiated DBA/2 alloantigen. L2 cells proliferate in response to alloantigen, but do not express cytolytic activity, whereas L3 cells, which do not proliferate in response to alloantigen unless exposed to 2^0 MLC or LCA supernatant fluid, express cytolytic activity specific for the original stimulating alloantigen. The magnitude of the cytolytic activity seems to be related primarily to the extent of L3 proliferation. The results observed in cultures prepared with L2 and L3 cells together with alloantigen are consistent with the interpretation that alloantigen stimulation of L2 cells results in proliferation and in the release of one or more soluble amplifier factors which bring about the proliferation of L3 cells with a concomitant increase in cytolytic activity. In preliminary experiments, supernatants have been obtained after 2 d from cultures of L2 cells stimulated with alloantigen. L3 cells grown in the presence of 'L2-cell supernatant fluid' together with irradiated allogeneic cells generated cytolytic activity that was 12 times greater than that observed in the absence of 'L2 cell supernatant fluid'. The availability of separate T-cell lines with amplifier or cytolytic activity will simplify the study of interactions involved in the development of cell-mediated immune responses.

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Association of a B-cell alloantigen with susceptibility to rheumatic fever

THE possibility that genetic factors determine susceptibility to rheumatic fever following infection with Group A streptococci was suggested by epidemiological and family studies¹. However, with the exception of a slightly increased frequency of Lewis group secretors², the identification of a genetic marker in the patient group has been unsuccessful. In particular, the distribution of HLA-A and B allotypes did not reveal any consistent difference from controls³⁻⁵. We report here that a novel B-cell alloantigen has been found at a significantly increased frequency among patients with rheumatic fever, providing further evidence for the value of B-cell alloantisera as reagents for the demonstration of disease associations with genetic determinants relevant to the immune system. This alloantigen was of particular interest because it was not found to be associated with any recognised HLA-D locus determinant.

The observation that B lymphocytes preferentially express differentiation alloantigens has led to a new approach to the use of genetic markers that might be of special significance to the immune system⁶. These are recognised primarily with alloantibodies that develop as a result of immunisation with paternal antigens during pregnancy. The majority of such alloantisera recognise alloantigens that have extensive biological and chemical homologies with the I-region antigens of the murine histocompatibility system and have been termed 'Ia-like' or simply 'Ia' (ref. 7). Some of these Ia alloantigens are closely related to HLA-D alleles defined in the mixed lymphocyte culture (MLC) reaction, and have been designated HLA-DR. Other B-cell alloantigens are not as readily related and seem to be primarily significant in demonstrating the immunogenetic associations of particular diseases^{8,9}.

Mononuclear cells were isolated from 61 patients with documented rheumatic fever (Jones criteria) attending clinics either in Bogota, Colombia, or New York City, and from age, sex and race matched control individuals. Reagent B-cell alloantisera were selected for their very low T-cell reactivity and further absorbed with pooled platelets. Two assays for B-cell alloantigens were used: mononuclear cells were stimulated with pokeweed mitogen for 4-6 d before staining using an indirect immunofluorescent technique¹⁰, and 'B' cells isolated by depletion of T lymphocytes were used in a modified Amos two-stage microcytotoxicity test⁹.

Twenty-eight lymphoblastoid lines were derived from individuals known to be homozygous for defined HLA-D alleles; 17 were derived from unselected individuals who were defined for all HLA determinants and known to be heterozygous for HLA-D. DR specificities were assigned according to the pattern of reagent serum reactions with the reference cell lines⁹. In the case of sera which detected an alloantigen jointly expressed on cell lines derived from individuals with different HLA-D alleles such as Dw4, Dw7 or Dw10, the specificity of the sera was designated $4 \times 7 \times 10$ (ref. 9). The χ^2 statistic included

Table 1 B-cell alloantigen typing in patients with rheumatic fever

Reagent serum		New York series (%)		Bogota series (%)		Combined series	
Designation	DR Specificity	Normal (52)	Rheumatic fever (21)	Normal (60)	Rheumatic fever (41)	χ^2	Relative risk
883	Undefined	17	71	16	75	50.144	12.94
1,818	1	31	20	24	20	0.906	0.686
1,558	2	28	33	20	30	0.878	1.463
1,033	3	24	25	20	15	0.745	0.68
924	4×10	26	25	20	30	0.463	1.26
1,038	4×7×10	28	35	32	40	1.397	1.54
1,995	5	12	0	10	15	0.125	1.21

the Yates correction for continuity. The relative risk was calculated according to Woolf¹¹ as equal to (patients positive/patients negative) × (controls negative/controls positive). Values greater than one indicate the degree of positive association of a marker with disease.

Reagent serum 883 was identified from among over 200 unselected B-cell alloantisera through its reaction in preliminary studies with a high proportion of patients with rheumatic fever. This and other sera with established DR specificities were used to determine their alloantigen frequencies in two populations of patients with rheumatic fever (Table 1). Serum 883 reacted with 75% of the patients in the Bogota series and 71% of the patients in New York, whereas the frequency of reaction in the two control populations was 16 and 17%, respectively. The association of alloantigen 883 defined by serum 883 with rheumatic fever for the combined series was significant at $P < 0.001$ and the relative risk was 12.9. We have not shown the unremarkable frequency of reaction with serum 883 among patients with a variety of other diseases, including tuberculosis, leprosy, post-streptococcal glomerulonephritis, multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus.

The expression of a number of Ia-like alloantigens, including some which were related to HLA-D specificities, was studied with 19 B-cell alloantisera. The frequency of alloantigens detected by these reagents among patients was not significantly different from their respective control groups. The results for six sera with representative specificities are included in Table 1.

Attempts were made to define the specificity of alloantiserum 883 using 28 B-cell lines derived from individuals homozygous for the various MLC D-locus alleles (Dw1, Dw2, Dw3, Dw4, Dw5, Dw6, Dw7, Dw10 and Dw11) and selected for their suitability as typing cells. Serum 883 did not react with any of these defined homozygous cell lines. In contrast, both peripheral blood B cells and B-cell lines derived from 17 individuals known to be heterozygous at the D locus gave strong reactions with serum 883 in three instances. However, the reactivity could not be correlated with a defined MLC specificity. Similarly, using B lymphocytes from 52 normal subjects, no significant association of the presence of alloantigen 883 could be established with any HLA-A, B or D alleles.

The recognition of alloantigen 883 through the disease association with rheumatic fever emphasises the usefulness of directly screening disease populations with multiple antisera as a means of gaining insight into genetic factors that otherwise might not be as readily apparent. The interpretation that alloantigen 883 is an allele of a segregant series that is either a second Ia locus unrelated to HLA-D or a totally distinct B-cell locus is favoured by three observations: the absence of any relation of alloantigen 883 to a defined HLA-D allele; the lack of a reciprocal decrease of any DR alloantigens among the patient group that would have been expected if the DR alloantigens were alleles of alloantigen 883; the occurrence in certain individuals of alloantigen 883 as a third specificity in addition to two conventional and non-cross-reacting DR alloantigens and their associated HLA-D determinants.

The association of this B-cell alloantigen with rheumatic fever provides direct support for the concept that an immunogenetic factor is relevant to the development of rheumatic fever after streptococcal infection.

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NK cell-sensitive T-cell subpopulation in thymus: inverse correlation to host NK activity

NATURAL KILLER (NK) cells have been shown to have an important role in resistance to some tumours (see ref. 1 for review) and it has been suggested that these NK cells may represent a T cell and macrophage-independent surveillance mechanism² similar in principle to the theory proposed by Burnet³ and Thomas⁴. Recent observations have led to the speculation that NK cells may also have a physiological role *in vivo* as NK cells were closely associated with the rejection of bone marrow grafts^{5,6}. Although the target 'antigens' of NK cells have not yet been fully defined⁷, a wide range of malignant and non-transformed cells, including normal thymocytes¹⁷ and bone marrow cells⁸ are also sensitive to low levels of NK-mediated cytotoxicity. We provide evidence here to support the view that the NK cells may be involved in the control of normal T-cell subpopulations within the thymus.

Thymocytes from 2-week-old A/Sn mice were lysed in a 3 h ⁵¹Cr release assay with nylon wool passed CBA spleen cells

as NK effectors (Fig. 1). As shown below in Fig. 4, this lytic effect was dose-dependent at effector to target cell ratios below 25:1, whereas higher numbers of effector cells resulted in a marked decrease in lysis for unknown reasons. The active killer cell shared the cell surface characteristics of mouse NK cells (data not shown), and showed the same strain distribution as previously reported for mouse NK cells¹ (Fig. 3). Moreover, cold targets inhibition assays have shown that NK cells recognise the same target structure on A/Sn thymocytes as on the NK-sensitive tumour targets YAC-1 (data not shown). Low but significant levels of sensitivity to NK-mediated lysis were also exhibited by bone marrow, peripheral blood and spleen cells from 1–2-week-old A/Sn mice (Fig. 1). Conversely the organ distribution of NK reactivity in adult mice invariably showed that the thymus is the organ of least NK cytolytic potential (shown for strain CBA in Fig. 1) as previously reported⁹.

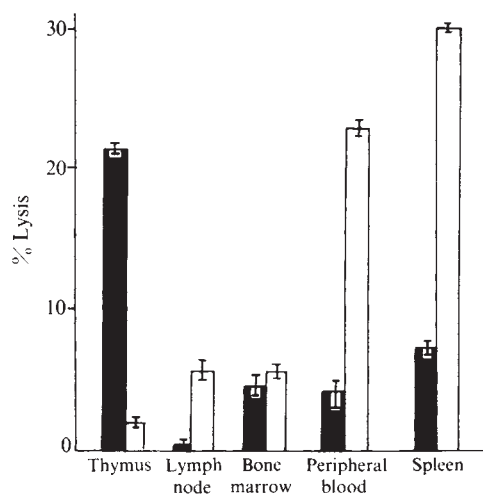


Fig. 1 Organ distribution of NK-sensitive cells in various lymphoid organs of 2-week-old A/Sn mice. Lymphocytes were enriched from peripheral blood by Ficoll-isopaque gradients followed by iron-magnet treatment to remove monocytes-macrophages. Spleen cells were treated with hypotonic shock followed by passage through nylon-wool adherence column to enrich for T lymphocytes. Cells from each organ were used as targets (solid bars) in a conventional 3 h ⁵¹Cr release assay using nylon wool-passed CBA spleen cells as NK effector cells at an effector to target cell ratio of 10:1 as described previously⁵. Open bars indicate organ distribution of NK reactivity in a standard NK assay using the YAC-1 tumour as target.

The NK sensitivity of thymocytes varied markedly with the age of the thymus donor. The highest sensitivity was observed with thymocytes from very young donors (<1–2 weeks) whereafter a gradual decrease of NK sensitivity occurred (Fig. 2). The mice that were used as thymocyte donors were also tested for their spleen NK activity. As shown in Fig. 2, the onset of NK activity around the third week after birth occurred simultaneously with a fall in the NK sensitivity of the thymocytes from the same mice. In old mice, NK activity as well as thymocyte sensitivity was low or absent.

The genetic control of thymocyte sensitivity was also investigated in several other strains of mice previously classified as high or low NK responders in extensive genetic studies (see ref. 1 for review). Again the inverse correlation between thymocyte sensitivity and NK activity was striking (Fig. 3). Thus, thymocytes from mice of low NK reactive strains (A, A.CA and AKR) displayed higher sensitivity to lysis than thymocytes from NK high reactive (CBA or DBA/2 × C57BL/6 F₁ hybrids) or mid reactive strains (C57BL/6 and DBA/2). Ten separate experiments yielded a mean lysis of 13.4% for A/Sn and 4.8% for CBA thymocytes ($P < 0.001$) from age-matched mice. The same relative differences in thymocyte sensitivity appeared when NK effector cells were taken from CBA, C57BL or A strains (Fig. 3). As high NK levels were invariably associated

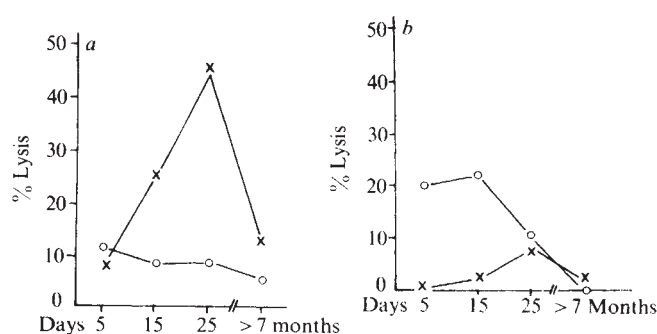


Fig. 2 Inverse correlation between the age dependence of splenic NK activity and the sensitivity of thymocytes to NK-mediated lysis. Thymocytes from three mice of each age were pooled and used as target cells in a 3 h cytolytic assay with nylon wool-passed CBA spleen cells as effector cells at an effector to target cell ratio of 50:1 (○). Spleen cells from the same mice were used as effector cells in a 3 h NK assay at an effector to target cell ratio of 50:1 with the YAC-1 tumour as target (×). *a*, Experiment with the CBA strain, *b*, with the A/Sn strain as thymocyte target and spleen cell effector donor.

with low levels of sensitive thymocyte targets it was important to determine the effect of NK boosting agents on thymocyte sensitivity in the same animal.

It is known that NK activity can be markedly augmented by interferon as well as several interferon inducing agents^{10,11}; all known to exert anti-tumour effects *in vivo*^{12,13}. When two such agents, tilorone (Fig. 4) and polyI:C (unpublished observation) were administered to young strain A mice a substantial increase in NK activity was apparent. However, thymocytes from these same animals were much less sensitive to NK-mediated lysis than thymocytes from controls, which again demonstrates the inverse relationship between levels of NK reactivity and thymocyte sensitivity to lysis.

There are various possibilities to consider when trying to explain this inverse relationship. The NK target distribution may be a direct consequence of the NK activity. Thus, in organs with high NK activity (spleen, peripheral blood, lymph node) or in individual mice of high reactive age or genotype, the natural target could simply be eliminated by the cytolytic effect of the NK cells, a process which would be enhanced by activation of the NK cells with tilorone. It is equally possible, however, that the NK-sensitive thymocytes and NK cells lie on different branches of a single differentiation pathway and thereby compete for a common progenitor. A third alternative is that

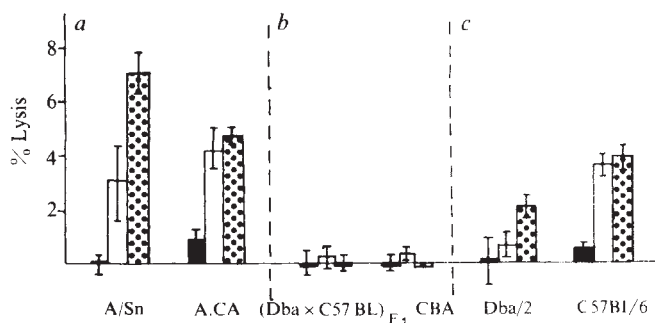


Fig. 3 Strain distribution of thymocyte sensitivity for NK lysis and its inverse correlation with genetically controlled NK reactivity. *a*, NK-low reactive strains; *b*, NK-high reactive strains; *c*, NK-intermediately reactive strains. Thymocytes were pooled from three mice at 3 weeks of age and used as target cells in a 3 h NK assay. Bar values represent the mean % lysis of triplicate samples $\pm$ s.e.m. Nylon wool-passed spleen cells from A/Sn (■), C57BL/6 (□), and CBA (▨) mice were used as a source of NK cells at an effector to target cell ratio of 10:1. These mouse strains were previously classified as to their genetically controlled NK reactivity¹.

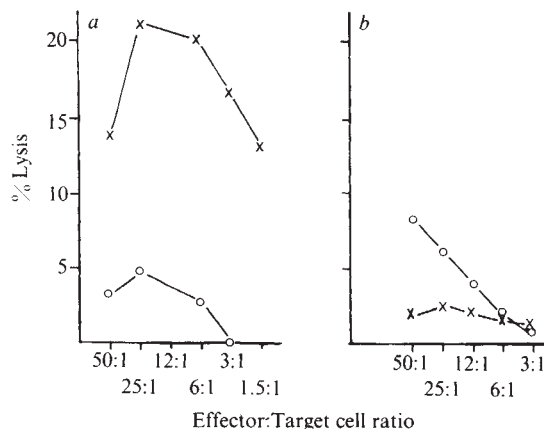


Fig. 4 Increased NK reactivity (*b*) and decreased NK sensitivity of thymocyte targets (*a*) following administration of the interferon inducer tilorone. Two-week-old A/Sn mice were each given 0.8 mg tilorone orally and 14 h later thymocytes from treated (○) or sham treated (×) littermates were tested as target cells in a 3 h cytolytic assay with nylon wool-passed CBA spleen cells as effectors (*a*). The total number of thymocytes recovered from tilorone-treated or control mice was 65 and 60×10^6 , respectively, indicating that the NK-sensitive population had not been eliminated by a cortisone-mediated effect¹⁶, which would have caused a marked cellular depletion of the thymus. *b*, increased NK reactivity in spleen cells from the same tilorone-treated (○) and control (×) mice as tested in a standard 3 h NK assay against the YAC-1 tumour target. The % lysis values are low as these mice are below optimum NK age (Fig. 2).

NK activity and the frequency of natural targets may both be regulated by a common extrinsic agent such as interferon. Trinchieri *et al.*¹⁴ have demonstrated that interferon can have opposing effects of increasing NK activity and decreasing target sensitivity for NK lysis. However, as the role of interferon in the age and genotype regulation of NK cells is not yet established, the two former explanations may be more likely. Preliminary results indicate that the NK-sensitive targets belong to the immature, cortisone-sensitive subpopulation of thymocytes. As NK cells are present in the bone marrow but not in the thymus, we will test the hypothesis that these 'precursor' T cells are destroyed in the marrow before, or during, their migration to the thymus.

In summary we have found an NK-sensitive subpopulation of T cells in the thymus. The frequency of these natural targets was inversely correlated to the level of NK reactivity in the thymocyte donor as judged by four separate criteria of organ distribution, age dependence, genotype variation and the effect of interferon inducers. Note that T-cell lymphomas in particular seem to be the most sensitive tumours for NK-mediated effects^{2,15}. It is an exciting possibility that the autologous NK target may in fact correspond to the target cell for leukaemic transformation and NK cells therefore may have a central role as a homeostatic control mechanism for this potentially neoplastic population.

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Activation of complement by cytoskeletal intermediate filaments

INTEREST in cytoplasmic intermediate (10 nm) filaments has grown since the successful immunohistochemical differentiation of these filaments from microtubules and microfilaments^{1,2}. Despite the widespread occurrence of intermediate filaments in various cell types, little is known about their function^{1,3,4}. A cytoskeletal nucleus-anchoring role has been suggested based on their intracellular distribution, their connections to the plasma membrane and nucleus, and their marked insolubility in detergents and salt solutions^{5,6}. The presence of autoantibodies against intermediate filaments in the sera of patients^{7,8} suggests that these filaments can be involved in pathogenetic processes. We report here that in studies on possible pathogenetic mechanisms involving intermediate filaments, antibody-independent binding of complement components to intermediate filaments has been observed. Initial observations were made using cultured human embryonal fibroblasts, as cytoplasmic filaments present in these cells have been characterised by using both immunological markers and drugs capable of selectively altering the morphology of microfilaments, intermediate filaments and microtubules^{1,7}. Subsequent studies showed complement binding to similar cytoplasmic structures of other cultured cells and also cells in tissue sections.

Cultured cells were made permeable to serum macromolecules by treatment with the non-ionic detergent NP40 (ref. 9) before incubation with serum. Complement components bound to cellular constituents were detected by incubation with fluorochrome-conjugated antibodies against human C1q, C4 and C3. Antibody conjugates detecting fibrin and immunoglobulins G, M and A served as controls. Direct immunofluorescent staining of fibroblasts incubated with fresh normal human serum revealed strong binding of the C1q subcomponent of C1 and of C4 and C3. A fine fibrillar cytoplasmic distribution of all three complement components was seen (Fig. 1a) using cultured fibroblasts originating from three different human fetuses. No binding of immunoglobulins or fibrin could be demonstrated. There was no staining if the detergent treatment was omitted, nor did staining occur with anti-complement conjugates alone. The complement binding phenomenon thus seemed to be independent of serum antibodies and could be demonstrated using 50 normal human sera without detectable antibodies against fibroblasts. Furthermore, purified C1q¹⁰ bound to cultured fibroblasts in a way similar to C1q of serum. C4 and C3 binding was inhibited in the presence of 10 mM EDTA and EGTA, which inhibit complement activation by chelating Ca^{2+} and Mg^{2+} (ref. 11). Binding of C1q occurred despite inhibition of C4 and C3 binding. The results indicate that C1q binding initiates Ca^{2+} - and Mg^{2+} -dependent complement activation by the classical pathway.

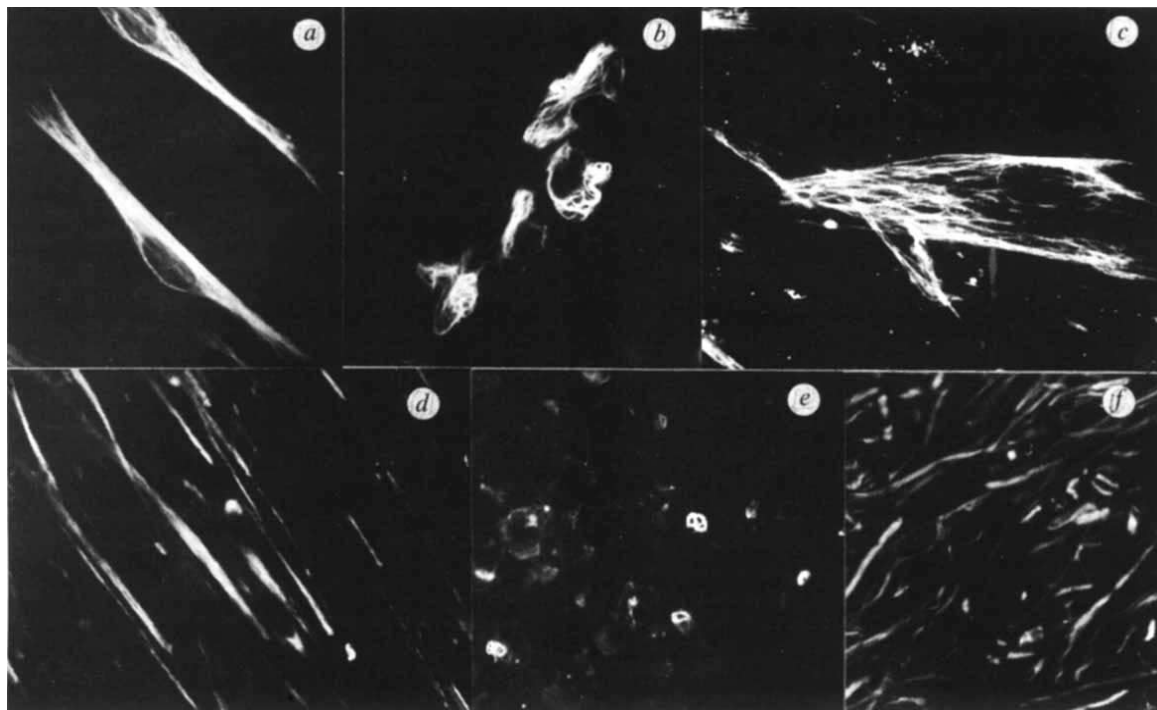


Fig. 1 Direct immunofluorescent staining of cultured cells (*a–e*) and human fetal connective tissue (*f*) demonstrating specific binding of complement components to cytoskeletal intermediate filaments. *a*, The cells were cultivated on coverslips in RPMI 1640 (Grand Island Biological) supplemented with 10% heated fetal calf serum (56 °C, 60 min), fixed in 3.5% phosphate-buffered paraformaldehyde to conserve cellular morphology, and treated with a 0.05% solution of the non-ionic detergent Nonidet P40 (NP40, BDH) to make cells permeable to serum macromolecules⁹. The cells were incubated with normal human serum (diluted 1:10 in saline) for 30 min at room temperature, washed in saline and treated with fluorescein isothiocyanate-conjugated rabbit anti-Clq conjugate (Behringwerke). *b*, Fibroblasts treated with vinblastine sulphate (Lilly, 10 $\mu\text{g ml}^{-1}$) for 4 h were similarly treated with serum and stained for bound Clq. Note typical perinuclear bundles of intermediate filaments decorated by bound Clq³. *c*, Fibroblasts treated with 0.1% detergent Triton X-100 (BDH) in saline for 15 min and then extracted with low and high ionic strength extraction buffers LAMES and HAMES⁶ containing 1 mM enzyme inhibitor phenylmethylsulphonylfluoride (Sigma) still exhibited an insoluble network of intermediate filaments attached to the substratum. This network retained the ability to bind complement. Binding of complement component C3 demonstrated after staining with TRITC-anti-human C3c (Behringwerke). *d*, Fibroblasts depleted of culture medium become permeable to serum constituents and exhibit irregular binding of Clq to intermediate filaments without pretreatment with detergent. Treatment otherwise as described in *a*. *e*, After incubation with serum, lymphoma cells K562 exhibit binding of complement component Clq to perinuclear fibrillar structures very similar to intermediate filaments in fibroblasts treated with microtubule disrupting drugs (*b*). *f*, Binding of complement components to cytoplasmic fibrils of fetal human mesenchymal cells was similar to the binding observed with cultured fibroblasts. Cryostat section of dermal connective tissue was treated as described in *a*. Magnifications $\times 500$.

The cytoplasmic structures binding Clq were readily identified. The localisation of bound Clq was indistinguishable from that produced by antibodies against intermediate filaments but was clearly distinct from that seen with antibodies against actin containing microfilaments or against microtubules^{3,7}. A typical change in the complement binding pattern, consistent with reorganisation of intermediate filaments³, was achieved by treating fibroblast cultures with the microtubule disrupting drugs, demecolchicine and vinblastine⁷ (Fig. 1*b*). Disruption of microfilaments³ with cytochalasin B did not alter the pattern of complement binding.

Intermediate filaments bound complement even after the cells had been subjected to treatment with non-ionic detergent Triton X-100 and subsequent extraction with high and low ionic salt solutions^{5,6} (Fig. 1*c*). Earlier studies have shown that these treatments leave only intermediate filaments in the cell residues, microfilaments and microtubules having been disrupted⁵. This further confirmed that Clq is bound to intermediate filaments and not to other cytoskeletal structures. Intermediate filaments able to bind complement were also demonstrated in nonviable fibroblasts permeable to serum proteins without detergent treatment, in cultures depleted of nutrient medium for 24 h (Fig. 1*d*). Preliminary studies have shown that cultured cells such as glia and glioma MG-251 cells bind complement in a similar way to fibroblasts, whereas perinuclear coiling bundles were observed in lymphoma cells K562 (ref. 12) (Fig. 1*e*). Further studies indicated that complement binding is not a property unique to cultured cells; this was shown most clearly in cryostat sections of fetal connective tissue (Fig. 1*f*).

The results suggest that Clq binding and complement activation through the classical pathway by intermediate filaments of different cell types is an antibody-independent recognition mechanism. Antibody-independent activation of Clq seems to be physiologically important and has been shown to operate against bacterial lipopolysaccharides¹³, virus-infected cells¹⁴, parasites¹⁵ and autologous cellular constituents such as heart subcellular membranes¹⁶, erythrocyte membranes¹⁷ and DNA¹⁸. Clq binding to intermediate filaments followed by classical complement pathway activation and accumulation of inflammatory cells¹¹ may be an elimination mechanism for poorly soluble cytoskeletal residues of damaged cells. It is tempting to speculate that failure of this mechanism results in chronic inflammatory reactions or extracellular deposition of intermediate filament residues in the form of morphologically and immunologically identical hyaline¹⁹ or amyloid^{20,21}.

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Diminished microtubules in fibroblast cells derived from inherited dystrophic muscle explants

MUSCULAR DYSTROPHY of the Duchenne type is inherited in a recessive, sex-linked fashion. The aetiology of the disease is still unclear but biochemical and morphological studies strongly suggest a cell surface membrane abnormality in Duchenne muscular dystrophy^{1–7}. Even though there are artefacts due to surface condensation of water, freeze-fracture studies of skeletal muscle from patients with Duchenne dystrophy show a depletion of intramembrane particles in the protoplasmic and environmental faces of the muscle plasma membrane^{8,9}. In contrast, Ketelsen's freeze-fracture studies indicated that the number of particles in the inner membrane fracture face in muscular dystrophy increased two- or threefold beyond normal¹⁰. In other studies, it was demonstrated that patients and carriers of Duchenne muscular dystrophy exhibited diminished cap formation in their lymphocytes^{11,12}. Although these experiments clearly support a membrane defect in muscular dystrophy, whether this is related to the aetiology of the disease is not yet clear. Several of these studies indicate that this membrane defect is an early and perhaps primary one in the aetiology of Duchenne's muscular dystrophy⁷. Even though the primary lesion in muscular dystrophy is usually thought to be

myogenic or neurogenic the reported abnormalities in the lymphocyte^{11,12} and erythrocyte membrane¹³ indicate that the expression of muscular dystrophy may not necessarily be restricted to these tissues. There have been many studies on the dynamic interrelationships between cytoplasmic structures and the cell surface^{14,15} and models have been proposed which suggest that changes in the state of the cytoplasmic microfilaments and microtubules can alter the mobility and distribution of membrane surface receptors¹⁶. In an attempt to determine if the membrane defect in muscular dystrophy might be correlated with a cytoskeletal alteration, we have studied the distribution of cytoplasmic microfilaments (actin) and microtubules (tubulin) in fibroblasts derived from muscle explants of both normal and dystrophic inbred chickens using indirect immunofluorescence methods with monospecific antibodies directed against actin and tubulin. We report here that fibroblasts from explants of cardiac and skeletal muscle of dystrophic chickens show a dramatic reduction in the appearance of intact interphase microtubules compared with those from normal chickens.

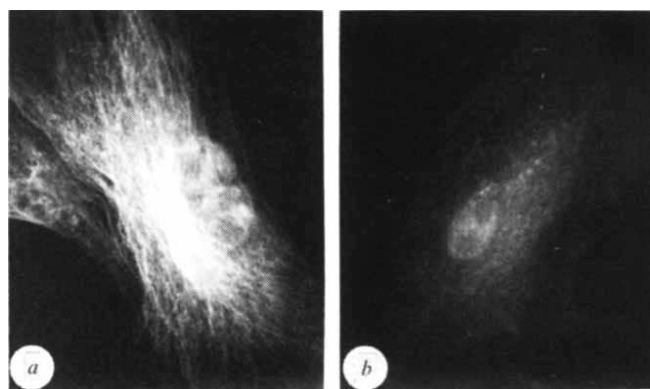


Fig. 2 Cells derived from normal cardiac explants (a) of a 7-d-old chicken possess full cytoplasmic microtubule complexes, whereas similar cells obtained from dystrophic cardiac muscle (b) possess diminished cytoplasmic microtubule complexes. Tubulin antibody stain. Scale bar, 10 μ m.

In our initial studies, we chose muscle explants from fertilised eggs and newborn chickens. The birds of line 413 are characterised by the onset of inability to right themselves about 3 weeks after hatching. The dystrophic chicken lines are considered to be a good model for human dystrophy because the progressive developmental onset of the disease resembles that seen in Duchenne's dystrophy. Line 413 birds are similar to the line 304 birds from which they were derived with regard to muscle fibre diameter, cytochemistry, acetylcholinesterase (AChE) levels, electromyography and serum enzymes¹⁷.

Small pieces (<1 mm³) of cardiac and skeletal muscle were placed on glass coverslips in 35-mm Petri dishes and allowed to attach firmly for 10 min before addition of 2 ml CMRL-1066 culture medium (Gibco) containing 10% horse serum and 5% fetal bovine serum. After 7 days incubation, a monolayer of cells grew out from these muscle explants which seemed morphologically to be primarily fibroblasts. In similar studies it has been reported that the cells which grow out from these muscle explants may in fact be aberrant precursors of myoblasts or an abnormal form of fibroblastic connective tissue¹⁸. The coverslips with attached fibroblast cells were rinsed in phosphate-buffered saline (PBS), fixed in 3% formaldehyde-PBS for 20 min, and then extracted in acetone at -10°C for 7 min. The cells were rehydrated from acetone in PBS and incubated with the antibody (0.1 mg ml⁻¹ for anti-actin; 0.05 mg ml⁻¹ for anti-tubulin) for 45–60 min at 37 $^{\circ}\text{C}$. Following four 15-min rinses in PBS, the cells were incubated in fluorescein-conjugated goat anti-rabbit IgA (Mely) diluted 1:6 with PBS. Finally, they were rinsed

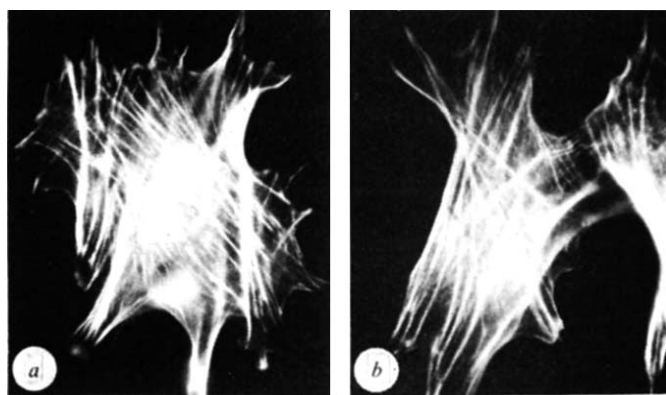


Fig. 1 Cells derived from cardiac explants obtained from 7-d-old normal (a) and dystrophic (b) chickens exhibit no apparent difference in morphology or distribution of cytoplasmic actin stress fibres. Actin antibody stain. Scale bar, 10 μ m.

Table 1 Presence of cytoplasmic microtubule complex in different muscle types

Muscle tissue type	No. of animals used	Age of animal	No. of cultures counted	No. of cells +CMTC	No. of cells -CMTC	Total no. of cells	% +CMTC
Normal cardiac	6	12-d embryo	8	577	203	800	72%
Dystrophic cardiac	5	12-d embryo	7	471	231	702	67%
Normal skeletal	6	12-d embryo	8	545	245	800	68%
Dystrophic skeletal	5	12-d embryo	7	507	193	700	72%
Normal cardiac	6	1-d chick	8	636	164	800	80%
Dystrophic cardiac	5	1-d chick	7	247	453	700	35%
Normal skeletal	6	1-d chick	8	583	235	818	71%
Dystrophic skeletal	5	1-d chick	7	238	462	700	34%
Normal cardiac	6	14-d chick	8	650	150	800	81%
Dystrophic cardiac	5	14-d chick	9	256	644	900	28%
Normal skeletal	5	14-d chick	8	650	150	800	81%
Dystrophic skeletal	5	14-d chick	9	229	671	900	25%

+CMTC, full cytoplasmic microtubule complex; -CMTC, diminished cytoplasmic microtubule complex. Normal control cardiac and skeletal fibroblast cells possess 60–80% +CMTC regardless of the age of the animal. The remaining control cells with a -CMTC are accounted for by those that are in mitosis, or other phases of the cell cycle in which the +CMTC is not apparent. Also, the cell population is not homogeneous and other cell types not containing full CMTC may be present. Fibroblast cells derived from dystrophic cardiac and skeletal muscle explants exhibit a loss in organisation of the cytoplasmic microtubules after hatching of the chick. This reduction of organisation and complexity of the CMTC becomes strikingly evident in the 14-d-old dystrophic chick.

four times for a total of 60 min and mounted on glass slides in a drop of glycerol: PBS (9:1 by volume at pH 9.5–10.0)¹⁹.

Figure 1 shows fibroblasts derived from normal and dystrophic cardiac explants obtained from 7-d-old chickens. These cells were stained with anti-actin to show the distribution of actin microfilaments and, as can be seen in Fig. 1, no differences are apparent. Figure 2 also shows fibroblasts derived from normal and dystrophic cardiac explants obtained from 7-d-old chickens which were studied for their microtubule distribution. The normal cells (Fig. 2a) contain a full complex of microtubules while the dystrophic cells (Fig. 2b) contain a diminished complex of microtubules. Figure 3 illustrates fibroblasts derived from normal and dystrophic skeletal muscle treated identically to those in Fig. 2. These observations indicate that there is a dramatic alteration in the distribution of intact interphase microtubules in cells derived from dystrophic tissue.

These differences in the distribution of microtubules cannot be accounted for on the basis of membrane impermeability to the tubulin antibody in the dystrophic cells for the following reasons: first, in the experiments with antibodies against actin, both the normal and dystrophic cells stained identically; second, the microtubules in the dystrophic cells are only diminished during interphase. The dystrophic cells grow at approximately the same rate as the normal cells and mitotic spindles composed primarily of microtubules seem to be identical by immuno-

fluorescence. Also, recent observations have indicated that rounded and flattened cell morphologies are variable in the expression of microtubules²⁰, but in this case, both the normal and dystrophic cells maintain a flattened morphology and thus, the diminished complex of microtubules in dystrophic fibroblasts does not seem to be related to overall cell shape. These results are consistent with a defect in the control of expression of interphase microtubule organising centres.

Other possible interpretations of these findings can be extrapolated from the recent work of others^{18,19}. Dise *et al.*²¹ reported results which indicated that an alteration occurs in the regulation of erythrocyte membrane function by calcium in human muscular dystrophy and Irish *et al.*²² have reported an alteration in the calcium binding component of the troponin complex (troponin-C) in avian muscular dystrophy. As microtubules are sensitive to intracellular calcium levels²³, our results could be interpreted to support a calcium alteration in muscular dystrophy.

In an attempt to analyse these findings further, a study of various stages of development was undertaken. As can be seen in Table 1, the diminished complex of microtubules is not present in fibroblasts derived from explanted cardiac and skeletal muscle obtained from embryonic material. Explants from 1-d-old dystrophic chickens show the first indication of loss of microtubules and this reduction is maximised approximately 2 weeks after hatching. These studies indicate that diminished microtubules in fibroblasts derived from inherited dystrophic muscle explants may be a progressive developmental defect and studies are now in progress to analyse the microtubules in lymphocytes and platelets from carriers and individuals with Duchenne's muscular dystrophy.

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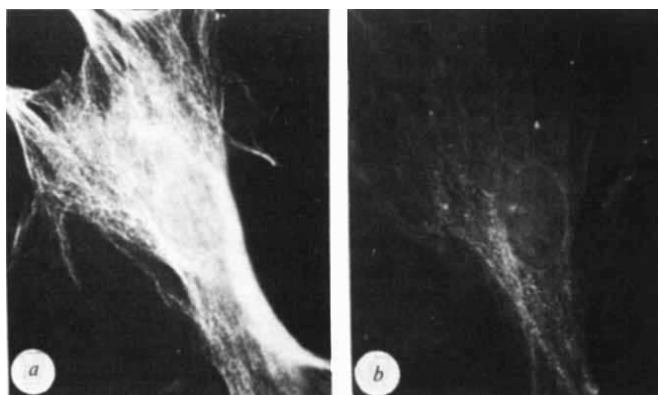


Fig. 3 Cells derived from normal skeletal muscles (a) of a 7-d-old chicken contain a full complex of cytoplasmic microtubules, whereas the cells obtained from the dystrophic skeletal muscle (b) possess diminished cytoplasmic microtubule complexes. These cells were prepared for immunofluorescence identically to those in Fig. 2. Scale bar, 10 μ m.

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Possible role of retinoic acid binding protein in retinoid stimulation of embryonal carcinoma cell differentiation

RETINOIDS, naturally occurring and synthetic analogues of vitamin A, are involved in the maintenance and differentiation of epithelial tissue^{1–3}. Furthermore, it has been reported that these compounds inhibit the growth of certain nontransformed and virally and chemically transformed cell lines^{4,5}. Recently, it has been shown that retinoic acid stimulates the differentiation of embryonal carcinoma cells^{6,7}, undifferentiated early embryonic-like cells present in teratocarcinomas. Embryonal carcinoma cell lines differ widely in their capacity to differentiate: a distinction has been made between 'pluripotent' and 'nullipotent' cell lines^{8,9}. In contrast to the latter, pluripotent embryonal carcinoma cells differentiate when injected into mice⁸ or when cell aggregates are formed *in vitro*¹⁰; some cell lines differentiate quite readily even when grown in monolayer culture. We have shown⁷ that retinoic acid not only stimulates the differentiation of pluripotent embryonal carcinoma cell lines, but also induces differentiation of the so-called nullipotent embryonal carcinoma cell lines nulli SCC1 and 6050 AJ. We show here that this effect is not limited to retinoic acid but that several other retinoids have the ability to stimulate differentiation of at least one embryonal carcinoma cell line, PCC4.aza1R. Furthermore, we present evidence to suggest that the action of retinoids may be mediated by a retinoic acid binding protein.

PCC4.aza1R embryonal carcinoma cells do not differentiate when maintained in the exponential phase or when grown to confluency; however, when cell aggregates are formed in a bacteriological dish and transferred to a tissue culture dish, outgrowth of differentiated cells is observed after a 2-d incubation period^{7,10}. The differentiated cells can be distinguished from PCC4.aza1R cells by their morphology and the production of plasminogen activator: outgrowths of PCC4.aza1R aggregates containing differentiated cells secrete plasminogen activator as indicated by the fibrin-agar overlay technique¹¹, whereas those containing no differentiated cells do not. The proportion of PCC4.aza1R aggregates giving rise to differentiated cells depends on the duration of incubation of the cell aggregates in bacteriological dishes. In standard conditions, at least 7 d of incubation are required before any PCC4.aza1R aggregates will produce differentiated cells; however, in the presence of 10^{-5} M retinoic acid, 100% of 1-day-old aggregates give rise to

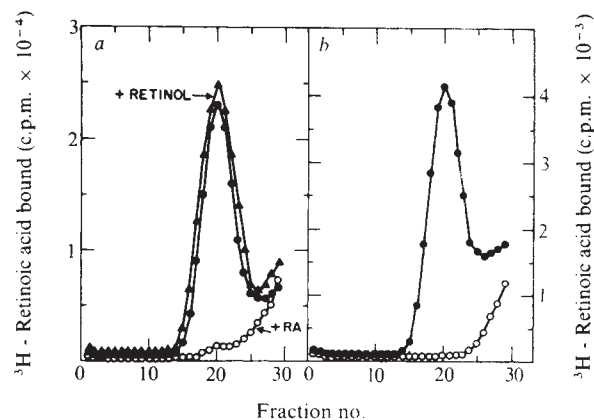


Fig. 1 Sucrose gradient analyses of the binding of ^3H -retinoic acid to cytosol and nuclear supernatants of PCC4.aza1R cell homogenates. Cells grown in monolayer culture in Dulbecco's modified Eagle's medium were collected and washed three times in phosphate-buffered saline (PBS). After resuspension in 10 mM Tris-HCl buffer (pH 7.4) containing 2 mM MgCl_2 and 7 mM β -mercaptoethanol, cells were homogenised with a Dounce homogeniser. Lysed cells were centrifuged for 5 min at 1,000 g. The supernatant was collected and centrifuged at 110,000g for 1 h. The resulting supernatant is referred to as the cytosol fraction. The nuclear pellet was resuspended in 0.32 M sucrose plus 3 mM MgCl_2 and layered at the top of 1.7 M sucrose and centrifuged at 22,000 r.p.m. for 1 h in a SW27 rotor. The resulting pellet contains purified nuclei. The nuclei were homogenised by sonication and centrifuged at 110,000g for 1 h. The resulting supernatant is referred to as the nuclear supernatant. *a*, The cytosol supernatant was incubated with 5×10^{-8} M ^3H -retinoic acid ($30.9 \text{ Ci mmol}^{-1}$) alone ($\bullet$), ^3H -retinoic acid plus 5×10^{-6} M unlabelled retinoic acid ($\circ$) or unlabelled retinol ($\blacktriangle$). Incubation mixtures were subjected to sucrose gradient centrifugation on linear 5–20% (w/v) sucrose gradients in PBS for 18 h at 220,000g. Fractions were collected and analysed for tritium content with a liquid scintillation spectrometer. *b*, Sucrose gradient analyses of nuclear supernatant of PCC4.aza1R cells incubated with ^3H -retinoic acid ($\circ$) and nuclear supernatant prepared after intact cells were incubated with 10^{-6} M ^3H -retinoic acid for 2 hr at 37°C ($\bullet$). Bovine serum albumin (68,000), ovalbumin (43,000) and myoglobin (17,000) sedimented in separate gradients at fraction numbers 3, 5, and 20, respectively.

differentiated cells^{7,10}. Using the aggregation procedure, we tested several analogues for their ability to stimulate differentiation of PCC4.aza1R cells (Table 1). In the control, (in the absence of retinoids), none of the PCC4.aza1R aggregates produce differentiated cells whereas in the presence of retinoic acid (no. 4) 100% of the aggregates differentiate. In general, the acid analogues are the most potent stimulators, although some (nos 12 and 16) are inactive.

A retinoic acid binding protein has recently been detected in the cytosol and nuclei of several tissues^{12–15}, and we have tested for the presence of such a protein in embryonal carcinoma cells. The 110,000g supernatant fraction of PCC4.aza1R cells readily binds ^3H -retinoic acid, demonstrating on sucrose gradient analysis a single, symmetrical receptor peak with a sedimentation coefficient of approximately 2S (Fig. 1a). A similar binding protein is observed in the cytosol of embryonal carcinoma cell lines OC 15 and nulli SCC1 (unpublished observations). Unlabelled retinoic acid competes with ^3H -retinoic acid for the binding site but retinol does not (Fig. 1a). Retinoic acid has a high affinity for the binding protein; an apparent dissociation constant of 2.3×10^{-8} can be calculated from the data in Fig. 2. No retinoic acid was detectable when isolated nuclei were homogenised and the supernatant fraction incubated with ^3H -retinoic acid. However, binding of retinoic acid was observed in nuclear fractions prepared from cells preincubated with ^3H -retinoic acid (Fig. 1b). This situation is very similar to that of steroid hormone receptors¹⁶, where nuclear binding is only detectable after hormone-receptor interaction in the cytoplasm.

Table 1 Comparison of the capacity of retinoids to stimulate the differentiation of embryonal carcinoma cells with their ability to compete for the binding site of the retinoic acid binding protein

Basic chemical structure	R	No.	% of aggregates differentiating			Competition for binding site
			10 ⁻⁵ M	10 ⁻⁶ M	10 ⁻⁷ M	
	CH ₂ OH	1	T	0	0	—
	CH ₂ OCH ₃	2	0	0	0	—
	CHO	3	50	0	0	—
	COOH	4	100	100	100	+
	COOCH ₃	5	0	0	0	—
	CH ₂ OCOCH ₃	6	100	10	0	—
	CH ₂ OH	7	0	0	0	—
	CH ₂ OCH ₃	8	0	0	0	—
	COOH	9	100	100	100	+
	COOC ₂ H ₅	10	0	0	0	—
	COOH	11	100	100	100	+
	COOH	12	0	0	0	—
	COOH	13	100	80	0	+
	COOC ₂ H ₅	14	0	0	0	—
	COOH	15	100	100	100	+
	COOH	16	0	0	0	—

Embryonal carcinoma cells (10^5 cells per dish) were added to Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 U ml^{-1} each of penicillin and kanamycin and $100 \mu\text{g ml}^{-1}$ streptomycin in a bacteriological dish (6 cm diameter). In these conditions cells do not adhere to the dish but proliferate and form aggregates. After 48 h incubation, aggregates were transferred to a tissue culture dish containing complete medium supplemented with the indicated retinoid dissolved in ethanol or with ethanol alone in the control (0.15% v/v final concentration). The aggregates attach to the dish and grow out. After 2 d of incubation the percentage of aggregates containing differentiated outgrowths was determined either on the basis of morphology or plasminogen activator production by the fibrin-agar overlay method¹¹. These criteria correlated very well with each other. Differentiation in control conditions (in the absence of retinoids) was zero. Analysis of competition for the binding site was carried out by incubating the 110,000g supernatant fraction, obtained as described in the legend to Fig. 1, with $5 \times 10^{-8} \text{ M } ^3\text{H}$ -retinoic acid ($30.9 \text{ Ci mmol}^{-1}$) plus 100-fold excess of the indicated retinoid for 1 h at 4°C . Competition was determined by sucrose gradient analysis as described in the legend to Fig. 1. Analogues 1 and 9 are retinol and retinoic acid, respectively; + and — indicate, respectively, the analogue's ability and inability to compete for the binding site. T, toxicity.

To investigate the possibility that the observed binding protein might be involved in the action of retinoids on embryonal carcinoma cells, the ability of the various retinoids to stimulate

differentiation of PCC4.aza1R cells was compared with their capacity to compete with ^3H -retinoic acid for binding sites on the retinoic acid binding protein. Table 1 shows that these two activities correlate well with each other. Retinyl acetate seems to be an exception because it does not compete for the binding site but is moderately active; however, this might be due to metabolism of the retinyl acetate into an active analogue. Modification of the cyclohexene ring of retinoic acid often does not interfere with the ability of the analogue to stimulate differentiation or to compete for the binding site, whereas substitution of the carboxylic acid group affects these activities severely. This suggests that the carboxylic acid group plays an important part in the interaction of these compounds with the binding protein.

The results presented here show that the induction of differentiation of embryonal carcinoma cells is not limited to retinoic acid but that several other analogues are also active. This activity correlates well with the ability of these compounds to compete for the binding site of the retinoic acid binding protein and suggests that their action on differentiation may be mediated by this binding protein. The results on the binding of retinoic acid to nuclear fractions indicate a similarity with the binding of steroid hormones to their receptors¹⁶ and suggest that retinoids may act as a mechanism similar to that of steroid hormones, that is, that complexes of retinoic acid and binding protein are transported into the nucleus, whence they modify gene transcription. However, on the basis of their observations that retinoids are involved in glycosylation reactions, De Luca *et al.*¹⁷ have proposed that retinoids may act through an altered biosynthesis of specific glycosylated intermediates. This hypothesis cannot as yet be ruled out.

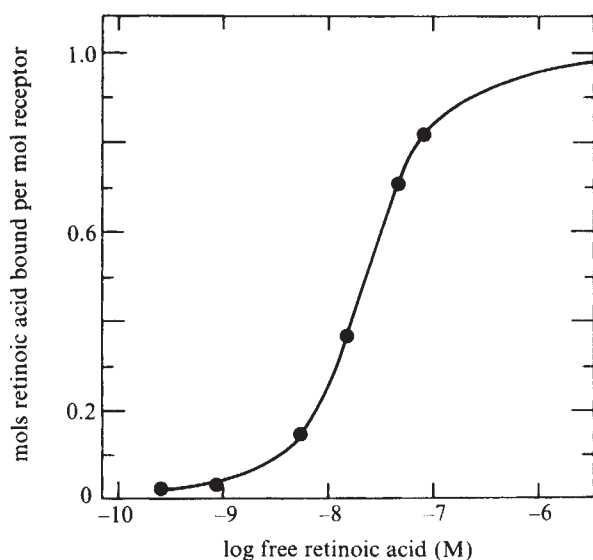


Fig. 2 Plot of ^3H -retinoic acid binding by PCC4.aza1R cytosol as a function of retinoic concentration. Binding assays were carried out as described in Fig. 1. The dissociation constant was determined according to the procedure described by Wiggert *et al.*¹⁴ and represents an average of the dissociation constants of all binding sites in the 2S fraction.

Several of the retinoids which are potent stimulators of differentiation of embryonal carcinoma cells are also very effective in reducing the occurrence and retarding the growth of certain tumours^{3,18-20}. The test described above might be useful as an *in vitro* screening procedure to identify those compounds with potential anti-tumour activity.

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Folate deficiency and decreased brain 5-hydroxytryptamine synthesis in man and rat

FOLATE deficiency was once considered to belong exclusively to the field of haematology. However, recently there have been reports of neuropsychiatric symptoms in folate-deficient patients¹⁻⁴. Among the most frequent symptoms are mild polyneuropathies, fatigue, mild depression and abnormal intellectual functioning. These symptoms, which can occur in the absence of any severe haematological changes at routine haematological testing, are responsive to folate therapy. Excess folate can also produce undesirable side effects. One study on the effect of pharmacological doses of folate (15 mg daily) had to be abandoned after 1 month because of side effects such as altered sleep patterns, malaise, irritability and overactivity⁵; the

same clinical complaints were found in some of our patients who received folic acid therapy and in whom folate levels were very high (M. I. B., unpublished data). In studies on rats, behavioural deficits were found in animals fed diets that were either deficient⁶ or over-supplemented⁷ with folate. Both active and passive avoidance behaviour was affected. In this study we have looked for biochemical changes associated with folate deficiency and over-supplementation. Because the regional distribution of 5-methyltetrahydrofolate in the brain is similar to that of 5-hydroxytryptamine (5-HT)⁸ we looked at the latter. We found that the concentration of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) is low in the lumbar cerebrospinal fluid (CSF) of folate-deficient patients who have folate-responsive neuropsychiatric symptoms. We also found low brain 5-HT levels in the brains of rats fed folate-deficient or over-supplemented diets. Thus, there is a close association between folate-dependent behavioural effects and low brain 5-HT.

Table 1 gives CSF data for controls and for patients with folate deficiency. All the folate-deficient patients had neuropsychiatric symptoms such as mild polyneuropathies, fatigue and mild depression and were selected according to diagnostic criteria described previously^{2,3}. The folate deficiency was caused by a long-lasting folate-deficient diet with or without decreased absorption of folate due to gastrointestinal disorders. The low levels of 5-HIAA in the CSF of the folate-deficient patients were not due to deficiency of precursor for 5-HT synthesis, as CSF tryptophan, which to a certain extent reflects the brain level⁹, was normal.

In two previous studies, one on psychiatric patients¹⁰ and one on patients with senile dementia¹¹, folate deficiency was not found to be associated with low CSF 5-HIAA. In a third study, anticonvulsant treatment was found to be associated with low CSF folate and high CSF 5-HIAA¹². The high CSF 5-HIAA was probably due to increased precursor availability, as CSF tryptophan was also high. The elevated tryptophan and 5-HIAA levels were clearly associated with blood levels of anticonvulsants which were within or above the therapeutic range. In the first two of these studies, CSF folate was measured in groups of patients, who were then divided into normal and low-folate groups for comparison of CSF amine metabolite levels. In the third study, the patients were grouped according to whether or not they were on anticonvulsant medication. Low folate levels are not usually associated with neuropsychiatric symptoms which are responsive to folate therapy^{1,2} so it is unlikely that there were many patients in these studies with folate-responsive signs. On the other hand, in our study, where folate deficiency is associated with low CSF 5-HIAA, the low-folate patients were selected on the basis of behavioural and neurological symptoms which respond to folate administration. This suggests that low CSF 5-HIAA is associated not so much with folate deficiency itself as with neuropsychiatric effects of folate deficiency, which are responsive to folate therapy.

Table 1 Folate, tryptophan and 5-HIAA in controls and folate-deficient patients

		Controls		Folate-deficient patients		P
		Mean $\pm$ s.e.	n	Mean $\pm$ s.e.	n	
Serum folate (ng ml ⁻¹)	L. Casei method	7.48 $\pm$ 1.61	5	3.26 $\pm$ 0.73	6	<0.025
	Radioactive method	6.11 $\pm$ 0.51	9	2.41 $\pm$ 0.35	7	<0.005
CSF folate (ng ml ⁻¹)	L. Casei method	22.3 $\pm$ 1.9	9	11.3 $\pm$ 1.6	7	<0.005
	Radioactive method	19.7 $\pm$ 1.3	9	8.8 $\pm$ 1.3	7	<0.005
CSF tryptophan (ng ml ⁻¹)		362 $\pm$ 57	9	280 $\pm$ 61	7	NS
CSF 5-HIAA (ng ml ⁻¹)		20.9 $\pm$ 2.4	8	10.3 $\pm$ 2.3	7	<0.005

CSF was taken by lumbar puncture in a sitting position at 9.00 a.m. Controls were patients suffering from neuropsychiatric disorders not known to affect brain amine metabolism and who had CSF taken for diagnostic purposes. The characteristics of the folate-deficient patients are described in the text. There was no significant difference between the ages of the control patients (mean $\pm$ s.d. 38.6 $\pm$ 16.4 yr) and the folate-deficient patients (50.0 $\pm$ 10.1 yr). The methods for determination of folate have been described previously². Tryptophan and 5-HIAA in CSF were measured by the method of Anderson and Purdy¹⁹.

Brain amine levels in rats given folate-deficient or over-supplemented diets are given in Table 2. Experimental conditions were exactly the same as those in earlier experiments in which folate deficiency⁶ and over-supplementation⁷ were found to cause deficits in active and passive avoidance behaviour. In both groups, noradrenaline and dopamine were unchanged, whereas 5-HT was low. These data strengthen the association between alterations of behaviour and brain 5-HT in folate deficiency and suggest that excess folate may have biochemical effects that are in some respects similar.

The mechanism of the effect of folate on brain 5-HT metabolism is unknown. The fact that the distribution of 5-HT in brain is similar to that of 5-methyltetrahydrofolate⁸ suggests that a methylation reaction plays an important part, but in spite of the varied metabolic actions of folate in brain¹³ there are no methylation reactions known to alter the brain 5-HT content. However, there is one other possible mechanism that could be tested. In rats folate deficiency can induce thiamine deficiency¹⁴ and thiamine deficiency is frequently found in folate-depleted patients¹⁵. Recently, it has been shown that rats on a thiamine-deficient diet develop an impairment of the cerebellar 5-HT system¹⁶. Thus, the influence of folate deficiency on 5-HT may be mediated by thiamine deficiency. However, another mechanism must be invoked to explain the effects of excess folate. Here, one possibility is an effect on the functional activity of tryptophan hydroxylase, the rate-limiting enzyme in 5-HT synthesis; this enzyme requires tetrahydrobiopterin (BH₄) as cofactor. The last step in *de novo* synthesis of BH₄ is the reduction of BH₂ and this step is carried out by tetrahydrofolate reductase¹⁷. Excess folate might competitively inhibit this enzyme, thus preventing formation of new BH₄ and decreasing the activity of tryptophan hydroxylase.

Table 2 The effect of folate on rat brain biogenic amine content

Diet	5-HT	Dopamine	Noradrenaline
Purina	342 ± 11	897 ± 32	634 ± 35
Control (folate deficient + 2 µg folate per g diet)	346 ± 26	954 ± 36	623 ± 20
Folate deficient	242 ± 36*	945 ± 42	702 ± 37
Folate excess (folate deficient + 100 µg folate per g diet)	238 ± 36*	990 ± 37	672 ± 71

Charles River CD rats (60–70 g) were randomly divided into 4 groups and placed on the diets shown above for 3 weeks. To avoid diurnal changes in brain amine content the animals were all killed in the morning. Brain amine content was measured by liquid chromatography with electrochemical detection (S.N.Y., unpublished method). A brain extract was prepared according to the method of Ahtee *et al.*²⁰ except that the brains were homogenised in the presence of epinine, which was used as an internal standard. The sample preparation involves partial purification of the amines on a column of Amberlite CG 50. The amines are eluted from the column with 3 ml of 1.2 M HCl and 0.05 ml of the eluate was injected directly into the chromatograph (model LC-40; Bioanalytical Systems). Chromatography was carried out on a 2.1 mm × 50 cm column of VYDAC SCX pellicular resin with a buffer 50 mM in both acetate and citrate at pH 5.1. The amines were detected using a carbon paste electrode set at 0.8 V against the Ag/AgCl reference electrode. The amines were eluted in the order noradrenaline, dopamine, epinine, 5-HT. At a flow rate of 0.6 ml min⁻¹ each run took 20–25 min. All values are given as the mean of 10 determinations ± s.e. in ng ml⁻¹.

*Animals treated with diets containing no or excess folate have significantly less 5-HT than control animals ($P < 0.05$).

Although the mechanisms of these effects must remain speculative at the moment, we have shown that a vitamin deficiency can decrease synthesis of a neurotransmitter and influence behaviour in man. Folate deficiency and excess cause similar behavioural and biochemical changes in rats. In humans, the behavioural effects of these two conditions have some symptoms in common such as irritability and altered sleep patterns. Whether folate excess decreases brain 5-HT synthesis in man is unknown, but our data suggest that excess of even a water-

soluble vitamin such as folate can have detrimental effects. The data on folate deficiency is of interest in view of the relatively high incidence of folate deficiency in psychiatric patients¹⁸. In some patients decreased 5-HT levels due to folate deficiency may be an important cause of psychiatric symptoms.

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Fibrin ϵ -(γ -glutamyl)lysine crosslink acceleration by red blood cells

FIBRIN, the skeletal protein of thrombi, is derived from its soluble plasma precursor, fibrinogen. Fibrinogen has a molecular weight of ~340,000. It is composed of three pairs of subunit polypeptide chains, A α , B β , and γ , of molecular weights 68,000, 56,000 and 49,000, respectively, joined by disulphide bonds. The serine protease thrombin releases fibrinopeptides A and B from the amino terminal end of the A α - and B β -chains of fibrinogen to leave the α - and β -chains of fibrin monomer. Fibrin monomers then align by noncovalent interactions to give a fibrin polymer gel. Under the influence of thrombin-activated factor XIII (factor XIIIa, activated fibrin stabilising factor, plasma transglutaminase) and calcium ion, covalent ϵ -(γ -glutamyl)lysine crosslinks are rapidly formed between γ -chains of adjacent fibrin monomers to give the γ -dimer subunit of partially crosslinked fibrin¹. The γ -crosslinking transforms the clot into a covalently bonded polymer, rendering it insoluble in 3 M urea and other agents that disrupt noncovalent interactions². Extensive crosslinking of the α -chain to give the α -polymer subunit of fully crosslinked fibrin requires several hours in plasma and confers relative plasmin resistance to the clot³. We report here an increased rate and extent of α -crosslinking when red blood cells are present in the fibrin clot.

Fresh whole blood was collected in a 9:1 dilution of 3.8% citrate and 1 M ϵ -aminocaproic acid (EACA). Red blood cells

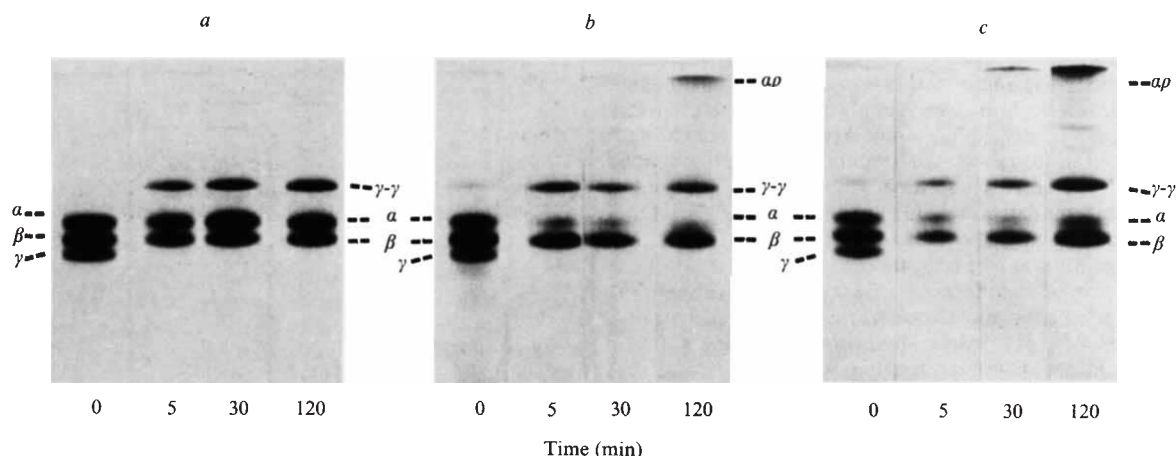


Fig. 1 5% SDS-polyacrylamide gel electrophoresis of reduced samples of a time course fibrin crosslinking study of clots from plasma (a) whole blood (b) and plasma plus RBCs (c). Crosslinking of α -chain to form α -polymer (α p) is greater when RBCs are present in clot.

(RBCs) and plasma were separated by centrifugation and red cells were filtered through a 1:1 mixture of microcrystalline cellulose and α -cellulose to remove white cells and platelets⁴. The red cells were then washed 10 times in 5 volumes of 0.9% NaCl, pH 6.5. The washed red cells were added to platelet-poor plasma from which they were separated to give a final haematocrit of 40–50%. Aliquots of 200 μ l whole blood, 100 μ l platelet-poor plasma and 200 μ l RBCs plus platelet-poor plasma were added to separate sets of four 3×50 mm glass test tubes. EDTA was added to a final concentration of 1% in the first tube of each set to prevent fibrin crosslinking. The mixture was clotted with human thrombin (Fibrindex, Ortho Diagnostics, or ultrapure thrombin) at a final concentration of 5 NIH units per ml. The resulting fibrin clot from the plasma sample was wound on a glass micropipette to express serum from the clot. Serum and red cells were expressed from the red cell containing clots between layers of filter paper. Clots were then washed three times in distilled water, dropped into 200 μ l of a solution of 2% mercaptoethanol–2% SDS–8M urea–0.065M Tris-HCl, pH 7.4, and placed in a boiling water bath for 5 min to stop the reaction.

Additional samples in each set of tubes were clotted with thrombin in the presence of calcium (final concentration of thrombin and calcium, 5 NIH units per ml and 80 mM, respectively). Samples were incubated at room temperature for 5, 30 and 120 min to allow fibrin crosslinking to occur before the fibrin clots were separated and processed as described above. As earlier studies had shown complete γ -crosslinking by 5 min, samples clotted for 5 min or longer were washed in 3 M urea to enhance removal of plasma proteins and red cells from the fibrin mesh.

Analysis of reduced samples by SDS-polyacrylamide gel electrophoresis⁵ revealed complete γ -crosslinking in clots of blood, plasma and plasma plus red cells by 5 min. The α -chain of fibrin crosslinked much more rapidly and extensively in whole blood and in plasma plus red cells than in plasma alone (Fig. 1). Disappearance of the α -chain was associated with appearance of the α -polymer. The presence of 0.1 M EACA inhibited plasminogen activation to plasmin and there was no build up of α -chain degradation products with time.

Gels stained with Coomassie brilliant blue were scanned using a Gelman ACD-15 densitometer. Areas under scans of the α -chain were compared with the sums of the areas under scans of the γ -chain, β -chain and γ -dimer to give a ratio (termed the α -ratio) of free α -chain to the sum of other fibrin chains. The α -ratio was plotted against time to quantitate the rate of disappearance of α -crosslinking. Such plots demonstrated that even extensively washed RBCs accelerated fibrin α -crosslinking in platelet-poor plasma to a rate and extent similar to that in whole blood.

To determine whether platelet factor XIII activity accelerated fibrin crosslinking in plasma, crosslinking studies were done as described above using platelet-rich and platelet-poor plasma. The rate and extent of fibrin α -crosslinking were not significantly different in platelet-rich and platelet-poor plasma.

In one of a series of studies designed to determine whether the release of intrinsic transglutaminase⁶ or other intracellular substance from red cells during clotting might be responsible for accelerated fibrin crosslinking, washed red cells were treated with *p*-hydroxymercuribenzoate (PMB) to block the sulphhydryl groups on their cell surface. Red cells were incubated in 5 volumes of 10 mM PMB in 0.9% NaCl, pH 6.5, for 20 min, and then washed three times to remove excess PMB. When these red cells were resuspended in plasma, they no longer accelerated the α -crosslinking of fibrin (Fig. 2). As PMB does not cross intact red cell membranes⁷, the crosslink-accelerating activity of RBCs must reside in or on the external surface of the cell membrane and be independent of intrinsic transglutaminase activity.

To further define the activity of red cell membranes in fibrin crosslinking, erythrocyte ghosts were prepared and their ability to accelerate fibrin crosslinking in plasma was determined. Erythrocyte ghosts prepared by hypotonic lysis⁸ did not accelerate fibrin crosslinking. As the fibrin crosslink accelerating activity of red cells was not diminished significantly by extensive washing in 0.9% NaCl, but was lost from hypotonically prepared red cell membranes, the activity must be due to a substance in or on the membranes that is inactivated or released by hypotonic media.

Additional experiments failed to relate intracellular red cell constituents to the acceleration of fibrin crosslinking by red cells. Glutathione in final concentrations of 1 mM, 3 mM and 10 mM had negligible effects on the rate of fibrin crosslinking in plasma

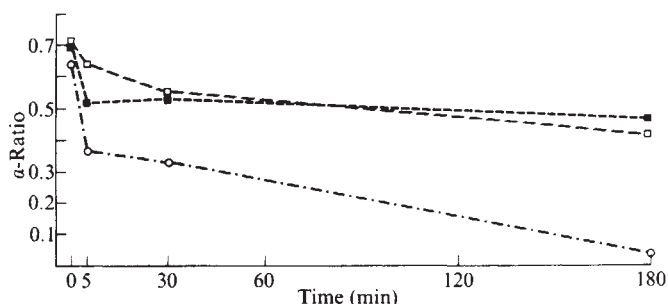


Fig. 2 Rate and extent of disappearance of α -chain in clots of plasma (□) plasma plus RBCs (○) and plasma plus *p*-hydroxymercuribenzoate-treated RBCs (■). The α -ratio reflects disappearance of free α -chain as this is crosslinked to form the α -polymer.

when compared with the effects of intact RBCs. The supernatant from red cell membrane ghost preparations also failed to accelerate fibrin crosslinking in plasma to the same extent as intact red cells. Generally, no difference in the rate and extent of crosslinking was observed, although some samples showed minimal (<10%) acceleration of crosslinking.

Because of the rapid rate of formation of γ -crosslinks, the effect of red cells on the rate of that reaction could not be adequately assessed. When the rate of crosslinking of the γ -chain of fibrin was evaluated at 4°C in plasma and in whole blood the reaction was still virtually complete at 5 min and no significant differences in the rate could be determined.

Our data are most consistent with the presence of a sulphhydryl-containing membrane protein located in or on the external surface of red cells that accelerates fibrin crosslinking. RBCs may thus have a more important physiological role in the acceleration of fibrin crosslinking and stabilisation of thrombi than previously recognised. Conclusions about *in vivo* fibrin crosslinking derived from studies of clotted plasma or fibrinogen may be misleading because of the absence of RBCs in such coagulum.

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Viroid replication is inhibited by α -amanitin

VIROIDS are infectious single-stranded circular RNA molecules¹ which cause disease in higher plants. Although the complete molecular structure of one viroid has recently been established², the mechanism of viroid replication and pathogenesis is still unknown. The viroid molecule carries insufficient genetic information to code for a complete viroid-specified replicase. Replication must therefore depend on host cell enzymes. We report here the inhibition by α -amanitin, of ³H-uridine incorporation into viroid RNA in infected tomato protoplasts. The inhibitory intracellular concentrations of α -amanitin which we determined suggest the involvement of the nuclear DNA-dependent RNA polymerase II in viroid replication.

Healthy plant cells contain two types of known RNA-synthesising enzymes, namely the DNA-dependent RNA polymerase system³ and the recently described RNA-dependent RNA replicase system^{4,5}. Viroids could therefore either be replicated through complementary RNA, as are most RNA viruses, or through a DNA template. This DNA template could be already present in the host cell in a repressed state or could be

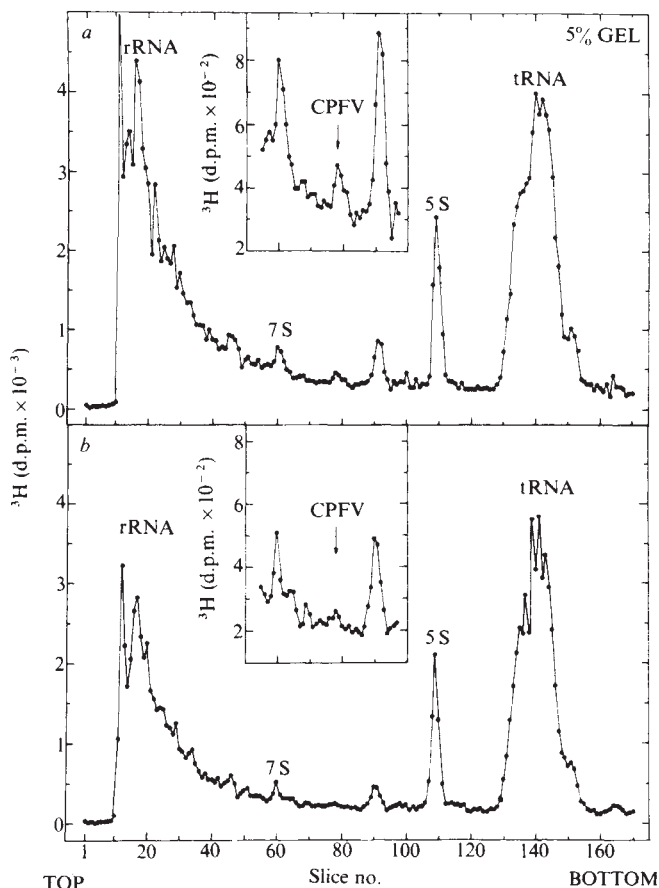


Fig. 1 Analysis of 2 M LiCl-soluble RNA from CPFV-infected tomato protoplasts in 5% polyacrylamide gel. Protoplasts were isolated from green leaves of the tomato cultivar 'Hilda 72' with the aid of Macerozyme R-10 and cellulase 'Onozuka R-10' at pH 7.0 and inoculated with 100 µg purified CPFV in 10 ml glycine-KOH-buffered mannitol at pH 9 containing up to 5×10^7 protoplasts¹⁰. After inoculation, protoplasts were cultured at a cell density of 2×10^5 ml⁻¹ at 29°C in the dark. ³H Uridine (10 µCi ml⁻¹) was added at the beginning of the culture period. 48 h after inoculation one-third of the protoplasts was collected by centrifugation, shock-frozen with liquid nitrogen and stored at -80°C until RNA extraction. To one-half of the remaining protoplast suspension α -amanitin (dissolved in culture medium) was added to give a final concentration of 50 µg ml⁻¹. An equal volume of culture medium without α -amanitin was added to the other half of the protoplast suspension. 72 h after inoculation, that is, 24 h in the presence of α -amanitin, all protoplasts were collected and frozen. RNA was extracted from these samples with the aid of phenol-SDS and salt-fractionated with 2 M LiCl⁸. RNA soluble in 2 M LiCl was dissolved in 200 µl buffer containing 8 M urea and aliquots of 50 µl RNA were analysed by electrophoresis on 16 cm long cylindrical 5% polyacrylamide gels. 0.1 A₂₆₀ of purified CPFV was added as marker and the gels were run at 1 mA per gel for 12 h at 7°C. After electrophoresis gels were stained with methylene blue, destained in 7% acetic acid, cut into 1 mm slices and counted for ³H radioactivity. *a*, RNA profile 72 h after inoculation and culture without α -amanitin. *b*, RNA profile 72 h after inoculation, but cultured in the presence of 50 µg α -amanitin per ml culture medium during the period 48-72 h after inoculation. The control data of ³H-incorporation into RNA between 0 and 48 h are given in Table 1.

newly synthesised on viroid infection. Previous studies with actinomycin D, which inhibits DNA-dependent RNA transcription, applied to leaf strips⁶ and nuclei⁷ prepared from viroid-infected tomato leaves indicated that viroid replication was unlikely to proceed through an RNA-dependent RNA replicase.

Tomato protoplasts isolated from green leaves of the tomato cultivar 'Hilda 72' as previously described⁸ were used. They regenerate new cell walls after 3 days⁹ and divide within a week (unpublished results). Their general metabolic capacity is substantiated by their ability to produce complete tobacco mosaic virus (TMV) particles after infection with TMV RNA *in vitro*⁹. In these protoplasts viroid replication has been followed by ³H uridine incorporation after infection *in vitro*¹⁰. Freshly

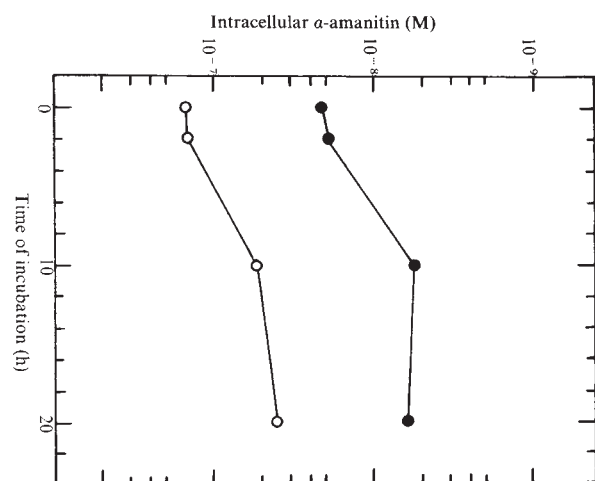


Fig. 2 Intracellular concentration of ^3H α -amanitin in tomato protoplasts. Protoplasts were isolated and cultured as described for Fig. 1. ^3H -O-methyl-dehydroxymethyl- α -amanitin (specific activity 2 Ci mmol^{-1}) was added to protoplast suspensions to a final concentration of $5 \mu\text{g ml}^{-1}$ ($\sim 10^{-5} \text{ M}$) (○) and $50 \mu\text{g ml}^{-1}$ ($\sim 10^{-4} \text{ M}$) (●). At 0, 2, 10 and 20 h of incubation with ^3H α -amanitin aliquots were collected, washed four times with culture medium, digested with Soluene (Packard) and counted for ^3H radioactivity. Quench corrections were performed in parallel experiments with equal numbers of protoplasts using ^3H toluene as standard.

isolated protoplasts were inoculated with cucumber pale fruit viroid (CPFV)¹¹ and cultured as previously described¹⁰. Control experiments comparing the effect of α -amanitin on TMV replication (which proceeds through an RNA-dependent RNA-replicase) were carried out on protoplasts of the same batch after inoculation with TMV RNA according to our published procedure⁹. Biosynthesis of cellular RNA, TMV RNA and viroid was measured by incorporation of ^3H uridine into these RNA species, which were separated on 2.5% and 5% polyacrylamide gels¹⁰ after extraction with phenol-SDS and salt fractionation⁸.

To discriminate clearly between the different DNA-dependent RNA polymerases possibly involved in viroid replication, we applied α -amanitin to our protoplast system. In eukaryotic cells α -amanitin selectively inhibits the DNA-dependent RNA polymerases II and III at low and high concentrations, respectively, whereas RNA polymerase I is not inhibited¹²⁻¹⁵. As shown in Table 1 α -amanitin has no appreciable effect on the biosynthesis of cellular RNA and viroid if present at a concentration of $10 \mu\text{g ml}^{-1}$ during the period 48–72 h after inoculation, when viroid replication is detectably increased¹⁰. With $50 \mu\text{g ml}^{-1}$ α -amanitin, viroid replication is inhibited to $\sim 75\%$, whereas the biosynthesis of tRNA, 5S RNA, 7S RNA and rRNA is again not appreciably affected (Fig. 1 and Table 1). We wish to point out that our protoplasts are killed by this concen-

tration of α -amanitin if present throughout the total time of culture. Therefore, only the brief exposure of protoplasts to this drug described above gives meaningful results. The RNA profile of uninfected protoplasts, where the viroid peak is naturally lacking, shows a similar effect of α -amanitin on the cellular RNA species.

In view of the concentration-dependent specificity of α -amanitin inhibition of RNA polymerases the interpretation of such *in vivo* experiments depends on knowing the intracellular concentration of this inhibitor. We therefore determined its intracellular concentration in our experimental conditions using a ^3H -labelled amatoxin¹⁶. When tomato protoplasts were exposed to 10^{-5} M ($50 \mu\text{g ml}^{-1}$) and 10^{-6} M ($5 \mu\text{g ml}^{-1}$) ^3H α -amanitin for 20 h, an intracellular concentration of $2 \times 10^{-8} \text{ M}$ and $2 \times 10^{-9} \text{ M}$, respectively, was already reached within the first 10 h incubation (Fig. 2). This clearly demonstrates that the intracellular concentration of α -amanitin is in the range of 10^{-8} M in the conditions in which viroid replication is inhibited, namely at $50 \mu\text{g}$ α -amanitin per ml culture medium. *In vitro* experiments with DNA-dependent RNA polymerases isolated from different sources¹⁴ including higher plants³ have shown that RNA polymerase II is, in fact, specifically inhibited at a concentration of 10^{-8} M α -amanitin, whereas inhibition of RNA polymerase III requires a 1,000-fold higher concentration¹⁵. Studies with animal^{17,18} and plant^{19,20} cells have shown that in comparable experimental conditions RNA polymerase II is also inhibited *in vivo*. As the intracellular concentration of α -amanitin which inhibits ^3H uridine incorporation into viroid RNA in our protoplasts is in the range where the nuclear DNA-dependent RNA polymerase II is specifically inhibited, we suggest that this enzyme system is involved in viroid replication.

Control experiments using tomato protoplasts inoculated with TMV RNA revealed that replication and accumulation of complete TMV was not affected, when α -amanitin was present at a concentration of $50 \mu\text{g ml}^{-1}$ during the period 24–48 h after inoculation, where TMV replication is known to increase logarithmically in our system (unpublished results). Therefore, the marked inhibition of viroid replication by α -amanitin is unlikely to be due to a secondary effect of this drug on cell metabolism in general.

For comparison, we also investigated the effect of actinomycin D on the DNA-dependent RNA metabolism in CPFV-infected protoplasts. As viroid replication in tomato protoplasts is known to increase to a detectable level 48–72 h after *in vitro* inoculation¹⁰, the inhibitor was applied so that it was present during this period. Actinomycin D at a concentration of $20 \mu\text{g ml}^{-1}$ culture medium inhibits total RNA biosynthesis up to $\sim 80\%$ (Table 2) which agrees with previous protoplast work²¹. The detailed analysis showed that viroid replication is inhibited to about the same degree ($\sim 85\%$) as the synthesis of cellular RNA. Identical results were obtained in control experiments with uninfected tomato protoplasts, the RNA profile of which differs only by lacking the peak representing ^3H -labelled viroid. From comparable results previously obtained with leaf disks⁶ and

Table 1 Effect of α -amanitin on RNA biosynthesis in viroid-infected tomato protoplasts

α -Amanitin $\mu\text{g ml}^{-1}$	Total RNA		rRNA		7S RNA		5S RNA		tRNA		CPFV	
	d.p.m.	% inhibition	d.p.m.	% inhibition	d.p.m.	% inhibition	d.p.m.	% inhibition	d.p.m.	% inhibition	d.p.m.	% inhibition
0	821,808	—	286,416	—	1,421	—	12,222	—	150,307	—	412	—
10	920,029	0	292,796	0	1,161	18.3	11,532	5.7	137,549	8.5	343	16.8
Control 0–48 h	224,800		62,120		1,450		3,155		32,487		181	
0	1,779,988	—	318,818	—	1,581	—	5,437	—	38,346	—	726	—
50	1,597,237	10.3	312,531	1.9	1,277	19.2	6,230	0	36,234	5.5	177	75.6
Control 0–48 h	1,664,876		353,212		333		5,198		47,395		31	

The figures are derived from experiments as described for Fig. 1. D.p.m. values given for total RNA and for each of the prominent RNA species indicate the incorporation of ^3H uridine into the RNA species during the period 48–72 h after inoculation, where α -amanitin was present. This net incorporation was calculated on the basis of the d.p.m. values of the ^3H -incorporation into RNA between 0 and 48 h after inoculation (control 0–48 h).

Table 2 Effect of actinomycin D and cycloheximide on RNA biosynthesis in viroid-infected tomato protoplasts

Actinomycin D $\mu\text{g ml}^{-1}$	Cyclohexi- mide $\mu\text{g ml}^{-1}$	Total RNA		rRNA		7S RNA		5S RNA		tRNA		CPFV	
		d.p.m.	% in- hibition	d.p.m.	% in- hibition	d.p.m.	% in- hibition	d.p.m.	% in- hibition	d.p.m.	% in- hibition	d.p.m.	% in- hibition
0	—	14,564,730	—	3,813,487	—	6,435	—	25,968	—	162,169	—	4,487	—
20	—	2,833,102	80.5	753,211	80.3	188	97.1	3,538	86.4	28,471	82.5	695	84.5
Control 0–48 h		5,646,928		1,438,744		1,335		12,334		138,961		108	
—	0	224,900	—	20,514	—	367	—	4,556	—	60,517	—	300	—
—	1	169,240	24.8	16,325	20.4	278	24.2	3,238	28.9	26,515	56.2	126	58.0
—	50	75,120	66.6	9,763	52.4	0	100	593	87.0	5,025	91.7	0	100
Control 0–48 h		84,856		7,892		163		1,213		22,438		41	

Protoplasts were inoculated with CPFV and cultured for 48 h as described for Fig. 1. At that time aliquots of the protoplast suspensions were collected by centrifugation and frozen. Actinomycin D or cycloheximide were added to remaining aliquots of the protoplast suspension to give the final concentrations as indicated. 72 h after inoculation, that is, 24 h in the presence of actinomycin D or cycloheximide, protoplasts were collected, RNA was extracted and analysed on 5% polyacrylamide gels. The data given show the [^3H]uridine incorporation into total RNA and into the different RNA species during the period 48–72 h after inoculation. This net incorporation was calculated on the basis of the d.p.m. values of the ^3H -incorporation into RNA between 0 and 48 h after inoculation (control 0–48 h).

nuclei⁷ from viroid-infected tomato leaves, the involvement of host DNA during viroid replication was inferred. However, as actinomycin D inhibits the biosynthesis of all RNA species including viroid RNA, a nonspecific secondary effect of this inhibitor cannot be ruled out completely. Therefore, we can exclude only the involvement of pre-existing RNA-dependent RNA replicases during viroid replication. These are actinomycin D resistant as demonstrated in several systems infected with conventional RNA plant viruses^{21–23}.

Finally, we studied the effect of cycloheximide on RNA synthesis to test the possible requirement of newly synthesised proteins for viroid replication. When cycloheximide is applied to CPFV-infected protoplasts in the range from 1 to 50 $\mu\text{g ml}^{-1}$ in exactly the same conditions as actinomycin D (Table 2), the biosynthesis of cellular and viroid RNA is strongly inhibited although cycloheximide is known to interfere specifically only with protein biosynthesis and not with nucleic acid transcription itself. Similar results are obtained with uninfected protoplasts. These results do not allow us to conclude that specific and newly synthesised proteins are a prerequisite for viroid replication, because cycloheximide exerts a nonspecific and rather general disturbance of cell metabolism.

The specific inhibition of ^3H uridine incorporation into viroid RNA by an intracellular concentration of 10^{-8} M α -amanitin strongly suggests that DNA-dependent RNA polymerase II is involved in viroid replication. In these conditions the biosynthesis of the prominent cellular RNA species (rRNA, 7S RNA, 5S RNA and tRNA) is hardly affected. RNA polymerase II is located in the nucleoplasm and is responsible only for the transcription of heterogeneous nuclear RNA species from the corresponding sections of the cellular DNA genome^{13,15}. Our assumption that the nuclear RNA polymerase II is involved in viroid replication is supported by earlier findings^{24,25} that the bulk of viroid infectivity is closely associated with nuclei and chromatin. Our present inhibition studies could explain the identical location of RNA polymerase II and of the accumulated viroid RNA as one of its possible products. The sensitivity of viroid replication to actinomycin D agrees with this conclusion irrespective of whether it is directly affecting a possible viroid-specific DNA template or is inhibiting DNA-dependent RNA biosynthesis in general.

The DNA dependence of viroid RNA replication has previously been postulated from hybridisation experiments with ^{125}I -labelled viroid RNA from which the existence of cellular DNA sequences complementary to viroid RNA in viroid-infected^{26,27} and even in healthy²⁷ host plant species was inferred. On the other hand, from a similar experimental approach viroid replication involving a complementary RNA was assumed²⁸. However, these conventional hybridisation experiments must be interpreted cautiously for the following reasons. The circularity and high degree of self-complementarity of the viroid molecule^{1,2}, require very specific experimental conditions to obtain reliable hybridisation data. Further-

more, the absence of any trace of contaminating cellular RNA species in the viroid probes used for hybridisation must be rigorously demonstrated by appropriate controls and by fingerprint analysis²⁹.

We emphasise that we do not regard the observed α -amanitin inhibition as definitive proof of direct replication of viroid RNA by the nuclear DNA-dependent RNA polymerase II. There are several possible mechanisms by which this enzyme system could be indirectly involved as a result of its central role in the formation of mRNAs in the host cell. There is also the remote possibility that viroid replication may proceed through a yet unknown pathway which is α -amanitin sensitive. If, however, DNA-dependent RNA polymerase II is directly involved it still remains to be determined whether viroid replication is based on derepression of a pre-existing viroid-specific DNA template or whether the infecting viroid molecule, because of its DNA-like secondary structure, is accepted directly as a template by DNA-dependent RNA polymerase II. The circularity and the unique secondary structure, which are a common feature of all viroids³⁰, are most probably of functional importance, and may add to the complexity of their replicating mechanism. For its elucidation, *in vitro* studies with appropriate enzymes, particularly of host plant origin, seem to be essential.

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Crystal structure of a eukaryotic initiator tRNA

OUR understanding of the molecular structure–function relationship in tRNA rests mainly on three types of information. First, on the common sequence patterns which have emerged from careful examination of many primary structures^{1–3}; second, a wide variety of spectral and other physical and chemical results must be accounted for by the molecular structure^{4–6}; and third, there is the detailed image of the yeast tRNA^{Phe} molecule independently determined and refined from two different—albeit similar—crystal forms^{7–10}. It is also clear, however, that the molecular model deduced from the yeast tRNA^{Phe} crystal structure cannot be easily reconciled with all structural requirements for function and is best considered a well-defined and stable canonical form of tRNA which is packed in an unusually well-ordered way in specific crystal lattices. Notwithstanding the enormous value of this canonical form in explaining the basic architectural features of tRNA, it is clearly important to image other crystalline tRNAs; particularly tRNAs that exhibit different functions (such as, initiators) or have significantly different covalent structures (for example, class III tRNAs)¹ or those that crystallise in different solvent conditions. We report here the initial results of the crystal structure determination of a eukaryotic initiator tRNA crystallised from a highly polar aqueous solvent^{11,12}. Its architecture is essentially the same as crystalline yeast tRNA^{Phe}, except for a small but significant difference in the position of the anticodon arm.

Our interpretation is based on a 4.5 Å electron density map prepared by the method of multiple isomorphous replacement (MIR) and augmented by direct methods¹³. The trace of the sugar–phosphate backbone is further constrained by four heavy-atom markers, each covalently linked to a specific residue and an elaborate network of crystallographic dyads upon which the

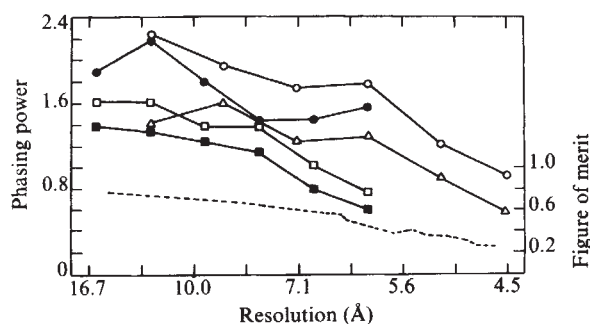


Fig. 1 Phasing power ($\sum |F_H|^2 / \sum (|F_H^{obs}| - |F_H^{calc}|)^2$)^{1/2} of five isomorphous derivatives plotted against resolution. They are gadolinium, lightly substituted, precession data (■); gadolinium, heavily substituted, precession data (□); gadolinium, oscillation data (△), pyridyl mercury acetate, precession data (●), and pyridyl mercury acetate, oscillation data (○). Figure of merit (---) is determined from MIR phasing³³ and has a mean value of 0.49 for all reflections to 4.5 Å resolution. F_H is the calculated heavy atom structure amplitude; $(|F_H^{obs}| - |F_H^{calc}|)$ is the 'lack of closure' error between observed and calculated isomorphous derivative structure amplitudes.

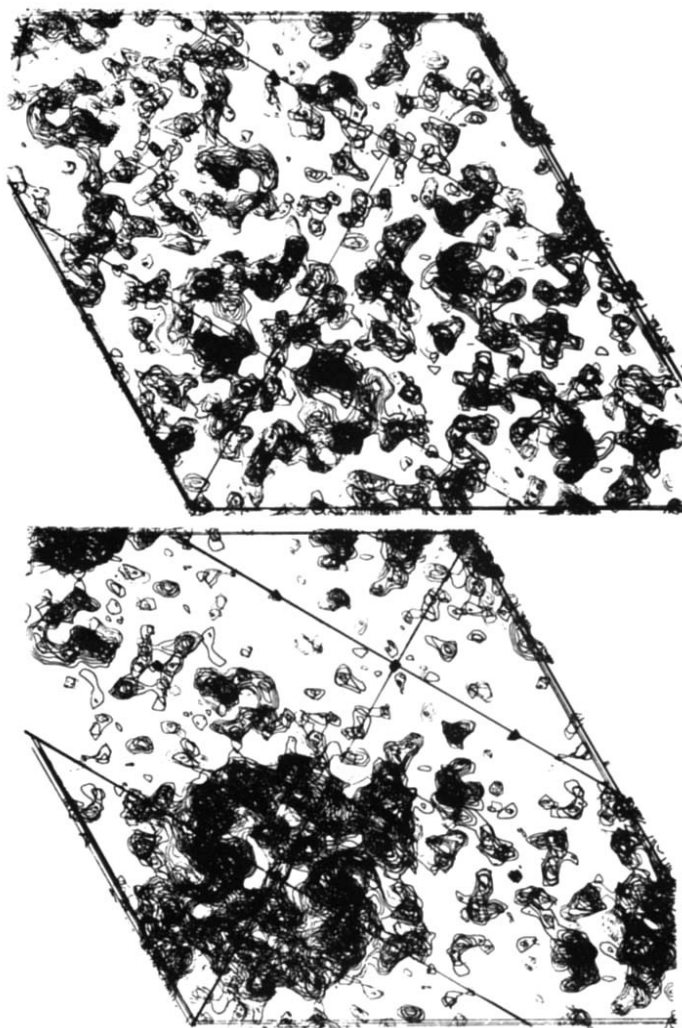


Fig. 2 Comparison of 10 sections of the 4.5 Å map of yeast tRNA^{Met} before (above) and after (below) the inclusion of 28 intense low resolution terms determined by matricial phase predictions. Both maps are contoured at the same intervals. Density cleared by this technique was ultimately shown to be noise, while density that was enhanced improved connectivity in the helical stems. Density on the dyad axes diminished.

molecule cannot intrude. The model presented here is based on a low resolution least-squares refinement procedure which assumes that the molecule is organised in four domains corresponding to the arms of the cloverleaf hydrogen-bonding pattern¹⁴.

The crystals have three important characteristics¹⁵. (1) They are grown from and stabilised in approximately 2M (NH₄)₂SO₄ containing 5 mM Mg²⁺ and 2 mM spermine and no organic solvent. The 2M ammonium ion may alter the structural role attributed to Mg²⁺ and spermine in crystalline yeast tRNA^{Phe} (refs 16–18). (2) Only 17.5% of the crystal volume is occupied by tRNA. Treating the remaining 82.5% as disordered solvent of uniform density was quite helpful in the structure analysis. (3) The crystal is poorly ordered, making accurate data collection very difficult and effectively impossible beyond 4 Å resolution. Data for the parent and three isomorphous heavy atom derivatives were collected initially to 6 Å by precession photography and extended to 4.0 Å by oscillation photographs in which a single crystal was devoted to each of ~30 overlapping 2.5° or 2° sectors. Figure 1 shows that there is useful phasing power in the best derivative to about 4.5 Å resolution. Schevitz *et al.*¹⁵ presented a 6 Å analysis of the yeast tRNA^{Met} crystal structure. Despite two heavy atom markers at iodine ⁵C₇₃ (5-bonding onto cytosine at residue 73) and osmium ⁵C₃₈ the electron density could not be convincingly interpreted in terms of the molecular backbone. Even when the data for the parent structure and MIR

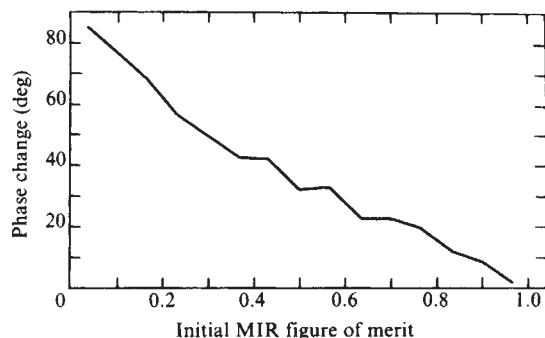


Fig. 3 Final phase change in degrees resulting from direct methods (see text) as a function of the original figure of merit determined by conventional MIR phasing. Phase information from both sources was merged by multiplying together their respective phase probability curves after each cycle of combined solvent levelling and density modification. Note that those reflections predicted to have a high reliability by MIR phasing (figure of merit near 1.0) remained closely tethered to their MIR phase while those with a low MIR reliability (figure of merit near 0) tend to be more easily shifted toward phases calculated by direct methods.

phases were extended to 4.5 Å resolution and a third heavy atom marker at iodine ^{125}I was used the map was still too 'noisy' to interpret confidently.

One particularly vexing deficiency in the MIR map was the inability to define the exact molecular boundary with

confidence. As over 80% of the unit cell volume was solvent this structure was particularly amenable to phase improvement by solvent levelling if the molecular boundary could be accurately established^{19,20}. The boundary was established by phasing the previously omitted very intense inner reflections ($d > 14$ Å). In the effort to secure high resolution data by extensively irradiating each crystal, these strong reflections were overexposed and far exceeded the dynamic range of even the most weakly exposed film. Even if relatively accurate amplitudes had been obtained for the parent and derivatives it is likely that the heavy atom contribution would not have been strong enough to provide accurate difference amplitudes to phase the very strongest of these reflections. To circumvent this problem the inner core of intense parent intensities was accurately remeasured and the phases of 28 of the very strongest reflections were determined by applying matricial methods to the amplitudes and MIR phases of 107 known reflections. The technique is similar to that used by Podjarny *et al.* to extend the phases of trileucine²¹ and yeast tRNA^{Phe} (ref. 22) to a higher resolution than the starting phase set. Here the procedure was used to extend phases to a lower resolution than the MIR phase set. The details of this work will be published elsewhere¹³; however, it is evident from Fig. 2 that there was substantial enhancement of the contrast between the molecule and the solvent. The improved map enabled us to confidently define 65% of the unit cell as solvent. New phases were calculated from a map in which the noise in the 'solvent' was levelled to a uniform average value and negative regions in the molecule attenuated^{23,24}. These phases were merged with

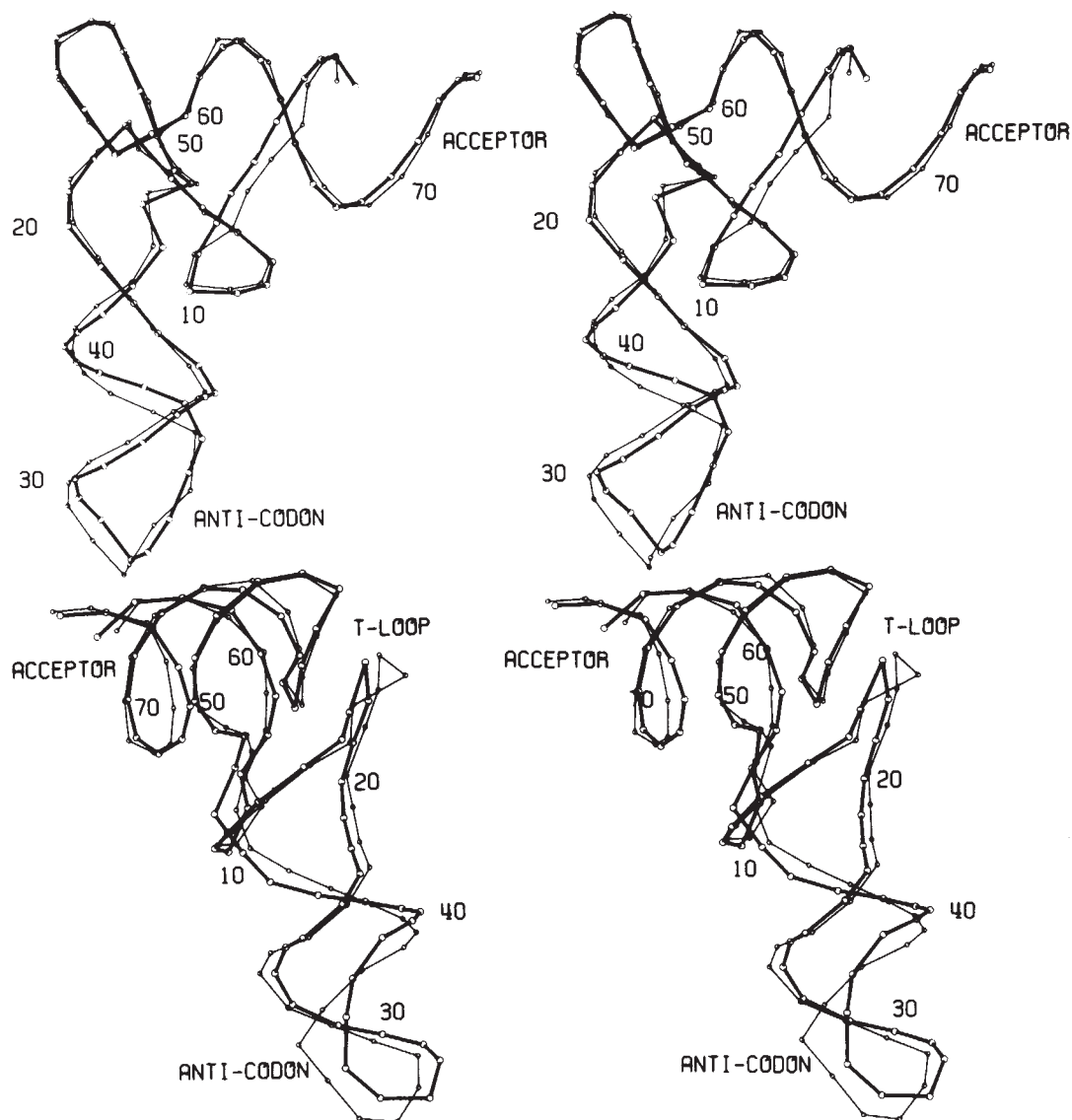


Fig. 4 Comparison of the yeast tRNA^{Met} (heavy line) and yeast tRNA^{Phe} (light line) structures in two stereo views. The phosphate backbone is shown with every tenth phosphate residue of yeast tRNA^{Met} numbered.

MIR phases and the 'density modification' procedure repeated for three cycles. Figure 3 shows that the phase improvement effected by density modification was most apparent for those reflections whose MIR phase was, in general, least accurate.

Four residues have been reacted stoichiometrically with covalently bonded heavy atoms. The heavy-atom markers were nicely distributed along the molecular backbone and provided a clear guide to the interpretation. (1) Iodine $^{5}\text{C}_{73}$: the third residue from the 3' terminus was labelled with iodine either by enzymatically rebuilding the 3' terminus with iodine ^{5}CTP and nucleotidyl transferase²⁵ or by directly iodinating the crystals²⁶. (2) iodine $^{5}\text{C}_{74}$: direct iodination also labels C_{74} which shows a small peak that cannot be refined, presumably because of disorder. (3) Iodine $^{5}\text{U}_8$: produced by direct iodination of the crystal²⁶. (4) Osmium $^{5,6}\text{C}_{38}$: direct reaction of the crystals with Os(VI) and pyridine²⁷ forming a 5,6-bispyridyl osmate diester^{28,29} with C_{38} (ref. 30).

Initially we interpreted the map with a conventional optical comparator and skeletal models, but it was found more convenient to use computerised 'static' molecular graphics³¹ to search for significantly different orientations of the stable helical substructures operating within the constraints imposed by the heavy atom markers and the crystallographic dyad axes upon which the model could not intrude. Subsequently, a residue-by-residue computerised fit of the entire model to the map was attempted in 'real time' at the Computer Graphics Laboratory at the University of North Carolina and this allowed us to rule out additional alternatives; however, we found the map was not sufficiently well resolved to impose a correspondence between phosphate, ribose and base moieties in the model and the peaks in the electron density.

The coordinates of the optimal fit were used as guides to position a tRNA^{Phe} model¹⁰ in the P6_422 unit cell. The root-mean-square deviation between the guide points and the tRNA^{Phe} model coordinates was 5.4 Å. This model was divided into four rigid groups corresponding to the helical arms and refined against observed intensities using a least squares procedure (CORELS) of Sussman *et al.*³² that 'restrained' the helical arms to remain within reasonable bonding distance of each other. The refinement was started at 12.5 Å and carried to 6 Å. The final crystallographic R factor between calculated and observed data to 6 Å was 42.3%; to 12 Å, 33%. These values indicate an essentially correct interpretation of a macromolecular crystal structure.

The molecular structure is shown in Fig. 4 contrasted with that of yeast tRNA^{Phe} . Except for a small region near the anticodon stem, the model accounts for virtually all the electron density in terms of a dominant sugar phosphate backbone. At this stage of the analysis the molecular architecture is generally similar to that of yeast tRNA^{Phe} .

The position of the anticodon arm in the crystal structure of yeast tRNA^{Met} differs significantly from the crystal structure of yeast tRNA^{Phe} (Fig. 4). Moreover, the refinement parameters show that although the anticodon arm converges to the position shown in Figure 4, it is the most poorly localised segment of the crystal structure. These observations suggest that the orientation of the anticodon arm is not rigidly fixed and the union of the D and anticodon stems may, as suggested previously^{18,34}, serve as a hinge point to allow alterations in the orientation of the anticodon during functional adjustments of the structure. Except for the possible stacking of a non Watson-Crick pair m^2G_{26} and A_{44} , no stabilising tertiary interaction can be invoked to ensure a particular orientation of the anticodon arm.

The molecules are packed with all 12 of the acceptor-T helices in the unit cell clustered around the 6_a axis at an angle of approximately 20°. Pairs of neighbouring molecules that have opposing acceptor stems stack upon one another roughly in helical register. This explains the intense continuous helical diffraction pattern along c^* observed at approximately 3 Å resolution, well beyond the lattice transform¹⁵.

The anticodon arms project out roughly perpendicular to the 6_a axes and contact neighbouring molecules at their anticodon

loop and the base of the D-stem. Thus many of the stabilising interactions involve the anticodon arm, which we believe to be extended on a flexible hinge. The fact that the lattice is connected through these hinged anticodon arms may be the reason that this (and possibly many other) tRNA crystals, although readily grown, are poorly ordered. The situation is reminiscent of the characteristically poorly ordered crystalline immunoglobulins³⁵—another macromolecule where flexibly hinged domains may be necessary for function.

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Note added in proof: Refinement with a more flexible model has reduced the crystallographic R-factor to 25% for all data to 4.0 Å resolution. Difference maps are clean and tertiary structural interactions are seen which can be correlated to those in crystalline yeast tRNA^{Phe} .

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A high molecular weight form of terminal deoxynucleotidyl transferase

CALF thymus terminal deoxynucleotidyl transferase (TdT) (EC 2.7.7.31) is a low molecular weight protein containing two peptides of MWs 24,000 and 8,000 in a native structure estimated to have a molecular weight of 32,000 (ref. 1). Use of antibody directed against homogeneous bovine transferase² for immunofluorescent cytology showed the normal thymus localisation to be cytoplasmic and nuclear, whereas bone marrow localisation is only nuclear³. Several human lymphoblastoid cell lines that contain terminal transferase activity also exhibit the nuclear location by immunofluorescence⁴. As cultured lymphoblastoid cell lines are readily labelled with radioactive amino acids we surveyed the peptides present in the immunoprecipitated transferase from these lines by fluorography of SDS gels. The results presented here show that a single 58,000-MW peptide is precipitated by monospecific rabbit antibody against bovine transferase. No peptides as small as those peptides present in calf thymus transferase were detected.

Our experiments used several lymphoblastoid cell lines. Line 8402 (ref. 5) contains >90% TdT⁺ cells and exhibits around 325 units of transferase per 10⁸ cells. Line 8392 (ref. 5), originating from the same patient, is completely negative by TdT immunofluorescence and enzyme assay. Line CEM-CCRF (ref. 6) contains about 19 units of enzyme per 10⁸ cells and has ~9% TdT⁺ cells. We cloned this line to produce CEM-clone 10 (>90% TdT⁺ cells, 280 units per 10⁸ cells) and CEM-clone 23 (<2% TdT⁺ cells, ~4 units per 10⁸ cells). All of these lines were grown in RPMI-1640 medium containing 10% fetal calf serum in spinner bottles at 37 °C. All have generation times of ~24 h in these conditions.

The human lymphoblastoid cells were labelled to high specific activity (about 60,000 c.p.m. per µg protein) by replacing the leucine and isoleucine in the medium with the corresponding ³H-amino acids. Cells were allowed to incorporate amino acids for 24 h and then extracts of the washed cells were prepared by sonication in 0.25 M potassium phosphate buffer at pH 7.5. The final cell extract is the supernatant fraction obtained after centrifugation at 100,000g for 1 h. Washed immunoprecipitates prepared from these cell extracts (see legend to Fig. 1) and separated on SDS-acrylamide gels produced the fluorograms shown in Fig. 1.

The results clearly demonstrate that a single radioactive protein is found in the immunoprecipitate from lines 8402 and CEM-clone 10 (Fig. 1, *a'* and *b'*). The MW of this peptide is estimated to be 58,000. No radioactive peptides were obtained from TdT-negative lines such as line 8392 (Fig. 1, *c'*) and negative clones such as line CEM-clone 23 (Fig. 1, *d'*). Positive lines regularly contained the 58,000-MW peptide and we conclude that this is a high MW form of human TdT. Mouse line P-1798 and human line NALM-1 (also TdT⁺) also contain the 58,000-MW peptide (data not shown).

The specificity of each of the antibody reagents used was carefully checked, and no nonspecific contaminants were found. This is demonstrated by the absence of bands in the transferase negative lines (Fig. 1, *c'* and *d'*). Our first experiments (not shown here) used a secondary goat (IgG) anti-rabbit IgG for complete precipitation of the rabbit anti-transferase-transferase complex and a large excess of goat heavy chain was therefore present on the SDS gel near the TdT peptide band. Although common practice indicates that this excess of high MW peptides does not affect migration of lower MW species, we reduced the amount of goat heavy-chain peptide on the gel by using the F(ab')₂ of goat IgG as the secondary antibody in the experiments presented in Fig. 1. The position of the 58,000-MW peptide is unchanged on SDS gels of the F(ab')₂ precipitated complexes. The radioactive band migrates slightly more slowly than the rabbit heavy chain arising from the primary antibody (Fig. 1, *a*, *b*, *c* and *d*), and the goat heavy-chain fragments

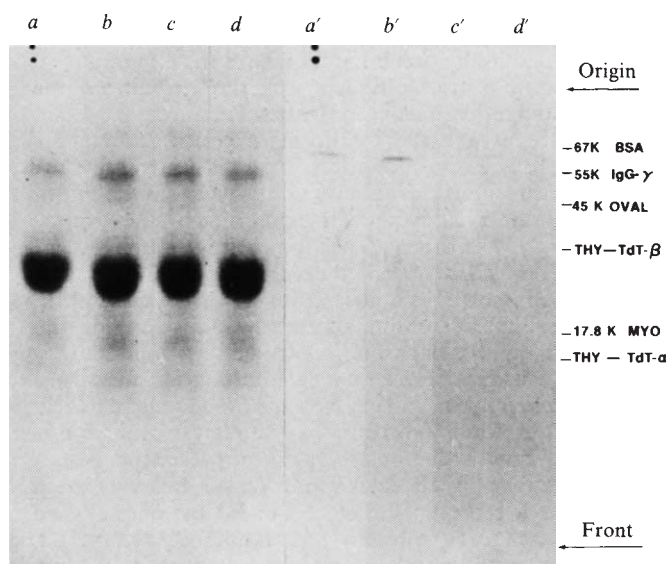


Fig. 1 Fluorogram of SDS-polyacrylamide gel of immunoprecipitated terminal transferase. Extracts of human lymphoblastoid cells labelled with ³H-leucine and ³H-isoleucine were precipitated with rabbit anti-bovine terminal transferase (0.36 mg ml⁻¹) purified by immunoabsorbent chromatography³ followed by F(ab')₂ goat anti-rabbit IgG (5 mg ml⁻¹). The immunoprecipitates were washed 4 times in Tris-Cl buffered saline, pH 7.2, and twice in Tris-Cl buffered saline containing 0.5% Triton X-100, 0.5% deoxycholate, 20 mM leucine, 20 mM isoleucine. The precipitates were resuspended for the final two washes by sonication. The washed precipitates were dissolved in sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.001% bromophenol blue, 5% 2-mercaptoethanol), heated for 1 h at 100 °C, and analysed on 16% SDS slab gels. Electrophoresis was carried out as described by Laemmli⁷. Gels were stained in Coomassie blue and processed for fluorography⁸. The position of MW markers and the α and β chains of calf thymus transferase are indicated by arrows. Lanes *a*, *b*, *c*, *d*, total protein in immunoprecipitates from line 8402, line CEM-clone 10, line 8392, and line CEM-clone 23, respectively (Coomassie stain). *a'*, *b'*, *c'*, *d'*, radioactivity from immunoprecipitates of line 8402, line CEM-clone 10, line 8392, and line CEM-clone 23, respectively. Peptide markers were bovine serum albumin (BSA, MW 67,000 67 K), human IgG (IgG, MW 55,000, γ chain), ovalbumin (OVAL, MW 45,000 45 K), calf thymus terminal transferase (THY-TdT- β and THY-TdT- α) and myoglobin (MYO, MW 17,800 17.8 K). The apparent MWs for TdT peptides in this gel system are 26,000 and 10,000.

migrate to the 24,000-MW region. Addition of pure bovine enzyme to the extract before addition of antibodies effectively competes with precipitation of radioactive protein. We conclude that the overwhelming majority of radioactivity in the immunoprecipitate has determinants related to the bovine enzyme.

The original work on rabbit anti-TdT demonstrated neutralisation of enzyme activity when the Ab-Ag mixture was assayed with d(pA)₅₀ and ³H-dGTP (ref. 2). A footnote suggested, however, that the immune precipitates could be demonstrated to contain enzyme activity. This was done by using an assay mixture containing d(pT)₃ and ³H-dATP to test for enzyme activity in the isolated immunoprecipitate. The small size of the initiator presumably permits diffusion into the immune complex and allows detection of enzyme activity at a level of around 10% of the free enzyme. We analysed enzyme activity in non-radioactive precipitates (requiring no background subtractions) and radioactive precipitates (which require rather large background subtraction) from line 8402 extracts and calculated that essentially all of the activity present in the original extract is present in the precipitate. We conclude that the 58,000-MW band on the SDS gels is responsible for the enzyme activity as no other bands are seen, and the precipitate is enzymatically active. The antibody procedure precipitates about 0.05–0.10 µg of protein from the crude extracts that contain

transferase activity, corresponding to 0.02–0.05% of the total cell protein added in the precipitation mixture. This amount of protein precipitate is also consistent with the level of enzyme protein estimated to be present in the extracts.

The existence of a high MW form of transferase in the malignant cell lines examined in this survey is a novel finding. Unfortunately this finding cannot be used to explain the multiplicity of forms observed by phosphocellulose chromatography of crude extracts^{9,10} of other tissues for the following reasons: Both line 8402 and CEM-clone 10 exhibit two distinct enzyme activity peaks on phosphocellulose (Hassur, Chang and F.J.B., unpublished results) but only a single peptide is seen on the fluorograms in Fig. 1; both enzyme peaks are inhibited by anti-TdT. The existence of several ionic forms of transferase in a single MW species requires an explanation based on amino acid changes or other ionic substitutions. Similarly, several isoelectric species can be demonstrated to be present in homogenous preparations of calf thymus transferase, but only the α and β peptides are present (Protzel and F.J.B., unpublished result). All species react with anti-TdT in microdiffusion tests. The immunoprecipitation procedures provide clean resolution of questions about peptide structure.

Our major objective in these experiments was to analyse a system exhibiting nuclear localisation of transferase. Having found a distinctly different form of transferase peptide in 'non-thymic' cells, we suggest that differentiation in the thymus may lead to peptide chain cleavage producing the 'thymic' form of enzyme. Of even greater speculative interest is the possibility that the high MW form, that form present in the nucleus, may be the form exhibiting important biological activity.

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Stopped visual motion

WHILE undertaking another experiment, we discovered a surprising and dramatic visual phenomenon. We were observing two gratings of high contrast and different spatial frequency (see Fig. 1 inset) while both gratings were rotating at a rate of 1 revolution per min. Both gratings were easily visible at the viewing distances used. The coarse grating was seen to revolve as expected; however, the fine grating appeared to revolve very slowly. This phenomenon has been shown to over 100 people¹ and all were startled by the observation. The individual reaction was to assert that the two gratings must be rotating at different physical speeds. This disbelief was readily corrected by removing the two gratings from their turntables and replacing each of them in the position of the other. Another entertaining variation of the observation is to observe the fine grating at the distance where it is perceived as a uniform disk; naturally, no movement can be seen at this distance. If one now advances slowly towards the grating it is initially seen as stationary; as one approaches closer it is seen to rotate progressively faster. At a particular distance it seems to rotate faster than the coarse grating. We have quantified these causal observations and show here that the

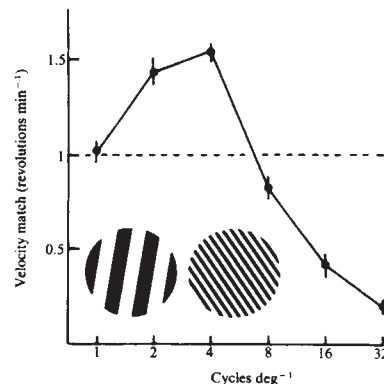


Fig. 1 Velocity match for gratings of different spatial frequencies—the dotted line is the true physical rate of revolution. The vertical bars are ± 1 s.e. ($n = 5$). The diameter of the disks was 10 cm.

perception of slow rotation movement is related to the spatial frequency and contrast of the pattern which is rotated.

A 1-cycle deg^{-1} grating was used as reference and it was rotated at 1 revolution per min. A second grating was attached to a variable-speed turntable which could be controlled by the subject. In the first experiment the subject was requested to look fixedly at one or the other turntable alternately. He then adjusted the speed of rotation until the two disks were matched in velocity.

The results are shown in Fig. 1. The horizontal dotted line is the 1 cycle min^{-1} reference velocity. It is clear that as the spatial frequency (fineness of the grating) is increased the apparent velocity rises, reaching a peak at 4 cycles deg^{-1} . Thereafter, the apparent velocity slows down rapidly.

Further quantitative observations established that the following variables affect the phenomenon. If the control grating is centred on the fovea and a grating of identical spatial frequency is viewed in the periphery of the visual field, the peripheral grating is seen to move much more slowly. In this experiment the subject looked with his fovea at a reference grating of 2 cycles deg^{-1} with a velocity of 1 rotation per min. With reference to the foveal standard grating the velocity match of a grating of identical spatial frequency at the following eccentricities were: 5°: 1.4 ± 0.2 ; 10°: 2.2 ± 0.2 ; 15°: 3.2 ± 0.2 ; 20°: 3.7 ± 0.3 ; 25°: 5.4 ± 0.2 .

At very low contrasts (near threshold) the perceived speed of rotation also slows down or stops. However, at medium and high contrast no change in the rate of rotation is found as a function of contrast.

The above observations suggest two separate mechanisms for detecting rotation. In the first, the motion is actually seen; in the second, no motion is seen, but if the subject looks for some time he deduces that rotation must be occurring for he remembers that some time before, the grating was, for example, at 12 o'clock and now it is at 3 o'clock, and so on. When he cannot see the real motion, the observer is left with the strange impression that somehow the continuity of the flow of physiological time is lost.

Neurophysiological studies on single neurones of the visual system are now required to establish whether this singular deception of motion is due to an insensitivity of visual neurones to rotary disks containing high spatial frequencies.

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reviews

A sociobiologist's new testament?

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Behavioural Ecology: An Evolutionary Approach. By J. R. Krebs and N. B. Davies. Pp.494. (Blackwell Scientific: Oxford, 1978.) Paperback £8.50; hard-back £18.

LARGE parts of this book are not really about ecology, and at least one chapter is not even about behaviour. Indeed, it might be more accurately (but still incorrectly) have been sub-titled *A Sociobiologist's New Testament*. The introduction says that it "takes modern population biology as its foundation and explores areas in which ethology and population biology overlap. Evolutionary thinking permeates the whole book", a statement of intent, which very clearly echoes E. O. Wilson's "Attempt to codify sociobiology into a branch of evolutionary biology and particularly modern population biology" (*Sociobiology: The New Synthesis* p4; Harvard University Press, 1975).

The text is divided into three sections, with an introduction (chapter 1) written by the editors. This chapter is an overview of ecology, natural selection and social behaviour, focusing particularly on the questions those who write later in the book will try to answer, and the tools with which they will answer them: optimality models, ecological stable strategies, and broad comparisons across species, together with brief summaries of the necessary genetics, the problems of altruism, inclusive fitness and group selection.

The first section contains four chapters, grouped round the theme "How animals harvest food, and how they avoid being eaten". Krebs discusses optimal foraging; Bertram the whys and wherefores of living in groups; Heinrich the economics of insect sociality; and Harvey and Greenwood anti-predator defence strategies, all topics that can properly be called behavioural ecology with the balance of the emphasis shifted towards one or the other of these words.

Part 2 (Sex, Mating and Signals) has five chapters with rather less unity. Maynard Smith under the heading "The Ecology of Sex" tackles the fascinating question of why organisms bother to reproduce sexually, with a brief look at parental care: excellent, no nonsense stuff which deserves (and has) a recent book to itself, but not

really ecology or even behaviour. Halliday in chapter 7 considers sexual selection and mate choice, followed by Parker who, as a colleague recently remarked, has the problem of mate choice "off pat" (for the uninitiated he studies dung flies). These two chapters complement one another well, as does Emlen's discussion of the evolution of cooperative breeding in birds. With less pleasing logic the section closes with a look at animal signals (by Dawkins and Krebs) under the pretext that the classical ethological work on signals was based on studies of sexual displays. The flavour of this whole section is very strongly behavioural, with only Emlen's chapter having much content that is strictly ecological, although this is intended as a signal of my own chauvinism rather than as a criticism.

The final section of the book tackles the problem of how an individual should deploy its behavioural options in space and time. Davies writes about territorial behaviour, and Partridge about habitat selection, both central themes in behavioural ecology. McCleery then carries us very firmly to the edges of modern behavioural theory with a chapter on how animals should make decisions about whether to mate, fight or feed, rather than what they should do within any one of these behavioural repertoires—the main concern of the rest of the book. Finally, Horn looks at life-history strategies, swinging us back to the mainstream of ecology with a chapter that contains some pleasing pot-shots at over-elaborate theory, some brilliantly simple insights, and almost no behaviour.

It ought, by now, to be clear that I found the book as a whole something of a mixed bag—an ecological, behavioural and sociobiological lucky dip—although like most lucky dips it is full of surprises and great fun to try. My feeling is that the editors have included just a bit too much. On the one hand there is material (for example, optimal foraging) that is certainly not sociobiology, and on the other, things that have precious little to do with mainstream ecology, or even behaviour. Perhaps we simply need a new pigeon-hole: but what should we label it?

With so many themes running

through it, it is difficult to provide one simple overview. Let me therefore close by developing three. As a teaching tool it will undoubtedly be valuable for students of all ages beyond first or second year undergraduate level. It is remarkably up to date, and covers well a host of complex ideas using hardly any mathematics (Parker's chapter is an exception in that he develops some formal models fairly extensively). Above all else it tends to avoid the glib phrase which hides a pit of uncertainty. Where there are problems it says so; and where there are exciting new roads to explore, it clearly signposts the way.

The book was presumably planned to complement R. M. May's *Theoretical Ecology*, which has the same publisher and format (for review see *Nature* 264, 138; 1976). Indeed, with the exception of Wilson's chapter in the latter, and Horn's chapter in the present book, there is almost no niche overlap between them. In combination they cover a massive area and might well be used together for advanced classes.

I suppose the real proof of a testament, old or new, is whether it will convert the hardened cynic: those who will argue that the whole edifice is but a harmless tautology. Throughout, behaviour is interpreted in terms of its survival value, what is in some sense 'optimal' or 'best'. And what is best? Obviously that which survives. The danger of slipping into this way of thinking is very real, because the sufficiently ingenious investigator can almost always dream up a convenient post-hoc Darwinian explanation for awkward observations, and without further testing, sit back contentedly having solved everything and nothing. Indeed there is more than a hint of this problem in several places throughout the book. For example, several authors make some very sweeping assumptions about the manner in which food 'must' be distributed in space and/or time, without actually measuring it, even though their original intention was to understand patterns of behaviour in relation to food supply. Even when ideas can be tested, they are difficult to refute because failure of the hypothesis can rather easily be attributed to "the rather artificial task with which [the animals] were faced" (p55); although

the artificiality of the situation can be conveniently ignored when the experiment 'fits' (for example, p44). However, and to be fair, a great deal of the experimentation and hypothesis testing is eminently reasonable. Indeed the editors have obviously anticipated most of the criticisms, and have been at pains both to draw attention to them, and avoid their worst excesses. What the book as a whole very clearly demonstrates is that if we accept the

central tenets of neo-Darwinism, and then use these tenets to generate interesting hypotheses and make predictions that can be independently tested, a great deal of behavioural ecology starts to make sense. At least, and on balance, it convinced this particular cynic. □

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Unity in the sensory systems

The Unity of the Senses: Interrelations among the Modalities. By L. E. Marks. (Academic: New York, San Francisco and London, 1978.) \$17.50; £11.35.

IN 1974 Dr Marks published a book on psychophysics and followed this a year later with an article on synaesthesia. In the present book he combines these two interests with his interest in nineteenth-century poetry. The theme which serves as a link, provides a title for the book.

The first three quarters of the book leads to the conclusion that the sensory systems are more than discrete information channels, they demonstrate some unity. The evidence upon which this conclusion is based is grouped under four headings each of which is, for Dr Marks, a doctrine. To quote his rather ill-chosen words "Each chapter therefore amalgamates one of the doctrines with the evidence that highlights it."

The first of these four chapters which form the core of the book, is concerned with the way in which the various senses cooperate in the perception of size, form, space, time, motion and number. The second explores the possibility that there are supra-sensory qualities—brightness is an example—shared by the sensory systems. The third doctrine states that different senses have similar psychophysical properties. Generations of students reared on a diet of Fechner, Weber and Stevens would not disagree. The doctrine of neural correspondence completes the quartet. It states that there is a neural analogue for each of the preceding psychological doctrines. In fact the chapter is mainly concerned with the way in which the sensory systems code intensity, but it does include some interesting philosophical icing.

These four chapters lead to the final chapter of the first part of the book, which turns out to be a very brief discussion of the possibility that the similarities between the senses are best

understood if each sensory system is considered as a modality of a general primitive sense from which it has evolved. The remaining part of the book provides many examples of how the unity of the senses reveals itself in the language of the poet.

I found this a disappointing book. First, it is badly written. Nobody should use language as carelessly as does Dr Marks. Even his definition of synaesthesia, a central concept, lacks precision. Second, the book is shaped by the author's psychophysical interest and expertise. The senses are analy-

sers, but (surprise!) they demonstrate some degree of unity. Perhaps this can be explained by their evolution from a more primitive sense. But should these signs of unity cause surprise? True the senses are analysers but because of their input into the reticular formation they are also very much involved with the integrative mechanisms of the body. The greater the degree of specialisation of the analysers the more complex and the more important are the integrative mechanisms.

Coghill and the Herricks with their partial patterns, which are concerned primarily with the analysis of experience and action, and total patterns, which are synthetic and which maintain the integrity of the living body, would have approached the problem differently. Perhaps the influence of their sort of biology would have provided a more useful foundation than the influences which led to the psychologist's psychophysics.

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Chemical kinetics

Principles of Chemical Kinetics. By G. G. Hammes. Pp. 268. (Academic: New York, San Francisco and London, 1978.) \$19.50; £21.50.

THIS book, which is intended for graduate and senior undergraduate students, provides a general introduction to chemical kinetics covering reactions in the gas phase, reactions in solution and enzyme kinetics. In dealing with such a wide spread of topics in a relatively small book the author has had to be selective in the examples chosen, but he has generally been careful to avoid oversimplification. In cases where the principles are illustrated by quite simple examples the nature of possible complicating factors is also pointed out and references are provided to more detailed treatments. The short but well-chosen reference lists at the end of each chapter provide valuable sources of supplementary material and allow the reader to pursue individual topics in greater depth.

The development of the material in this book has been well planned with the reader being led naturally from one topic to another. The balance between the theoretical and empirical aspects of the subject is also carefully maintained with the author taking pains to point out the difference between the

theoretically achievable and the practically possible. Emphasis is given to relatively recent developments in experimental technique and to areas of particularly active current research interest in a way that successfully captures some of the author's own enthusiasm for the subject. Not surprising, in view of the author's own fields of interest, the sections on fast reactions in liquids and transient phase enzyme kinetics are especially valuable in this respect. A particularly useful feature of this book is the carefully thought-out problems at the end of each chapter that provide a challenging test of the reader's understanding of the material presented.

It is perhaps a measure of success of this book in stimulating the reader's interest, rather than a criticism, that there are several sections that I felt could have been given more space, although not at the expense of any of the other material. From my own prejudiced viewpoint I would have liked to have seen more space devoted to enzyme kinetics because of the successful way in which the author relates this subject to its background in chemical kinetics rather than treating it as a topic in isolation. I would have no hesitation in recommending this book most highly as a lucid and stimulating introduction to its subject.

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Mycotoxins

Toxicology, Biochemistry and Pathology of Mycotoxins. Edited by K. Uraguchi and M. Yamazaki. Pp. 288. (Halsted/Wiley: New York and London; Kodansha: Tokyo, 1978.) £19.50.

"THE concept of mycotoxins is not yet widely appreciated" is a key statement in the preface to this book. To most laymen the notion that common moulds may produce potent animal toxins may be novel, but many industrial organisations involved in the production of human and animal foodstuffs are becoming well aware of mycotoxins. As is to be expected with a new branch of science which just happens to embrace one of the most carcinogenic substances, opinions vary from those who would attribute to mycotoxins several human and animal syndromes of unknown aetiology, to those who may have good commercial reasons to claim that there is little evidence that these substances have ever done any harm, particularly to humans.

The literature on the mycology, chemistry, biochemistry and pathology of mycotoxins has, in the past fifteen years or so, been evidence of one of the fastest growing facets of biological science. The first comprehensive review was included in the 1971-72 Academic Press series *The Microbial Toxins*. In the past year Marcel Dekker have published their three-volume *Encyclopedic Handbook* which, although it took several years to produce, is perhaps the most comprehensive current text. The present book is a useful and interesting addition to the review literature. It is unique in that it is written entirely by eight Japanese scientists, all of whom have published in the areas they review. It is natural, therefore, that there should be a thread of national emphasis through the book, in which the subject is treated mainly in the context of foodstuffs.

Western literature has tended to ascribe the origin of the recent wave of mycotoxin research to the 'Turkey X' syndrome in England during 1960. It is therefore salutary that the introduction to this book should emphasise the precedence of Japanese research into an epidemic of human acute cardiac beriberi in Japan between the two world wars. These studies led to the isolation of the yellowed rice toxins in the 1940s, though too late for direct verification of their involvement in the aetiology of cardiac beriberi—the epidemic by then having passed away, apparently, for good.

A notable feature of this book is the high standard of readability of the text. Several 1976 references are included, implying that the book can

claim to number among its 1,000 references most of the relevant studies available for review. It would be possible to instance a number of minor errors and omissions in the text but since mycotoxicology is expanding so quickly there is much to commend fairly rapid publication of a review-type book before it becomes too outdated.

The first chapter lists important mycotoxin-producing fungi and their significance concerning foodstuffs. The second chapter on the chemistry of mycotoxins is subdivided according to biosynthetic features, a treatment reminiscent of W. B. Turner's important 1971 book on fungal metabolites. The next three chapters are devoted to toxicology, the main topic of the book. There are sections on general and comparative toxicology. The fate, metabolism and mutagenicity of mycotoxins are discussed and cellular alterations, histopathology and carcinogenicity are liberally illustrated. As the chronic carcinogenic properties

of certain mycotoxins, notably aflatoxin, are of particular relevance to humans, the book concludes with a short chapter on traditional Japanese fermented foods, home-grown rice and imported peanuts and peanut products. Traditional foods are given a clean bill of health as the selection over many centuries of *Aspergillus* 'koji-moulds' for the preparation of miso, sake and soy sauce seems not to have included any capacity for aflatoxin biosynthesis. Further, the absence of aflatoxin from mouldy rice, otherwise contaminated with ochratoxin A and the aflatoxin precursor, sterigmatocystin, suggests that aflatoxin producing moulds are not indigenous to the relatively high latitudes in which Japan is situated.

This book is to be commended as providing a most useful oriental view of mycotoxicology.

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Physiology and pathology of the neutrophil

The Neutrophil: Function and Clinical Disorders. By S. J. Klebanoff and R. A. Clark. Pp. 810. (Elsevier/North Holland: Amsterdam, 1978.) \$148; Dfl.333.

IN recent years immunologists have focused their attention on macrophages and lymphocytes and minimised the importance of the neutrophil as a component of the cell-mediated defence system. This excellent book should help to redress the balance by stimulating interest in this fascinating cell by supplying a comprehensive account of contemporary knowledge of its physiology and pathology and a thorough citation of the literature.

In these days of specialisation a work as comprehensive as this, encompassing over 800 pages and possibly five times as many references, is generally tackled by a team of expert contributors. One of the strengths of this book is that the authors do not have vested interests in most of the contents and as a result the facts are presented in an unbiased manner without any attempt to influence readers' interpretation of the data. This has resulted in a highly informative exposition of current concepts that are expressed clearly and concisely. The mass of information that has been assembled is more suitable as a source

of reference than as an easily readable introduction to this cell.

A wide range of topics of cell biology and pathology have been systematically covered, including both normal and disordered structure, movement, phagocytosis, degranulation and antimicrobial systems of the cell as an entity, and its interaction as a component of the inflammatory process as a whole. The discussion of clinical disorders of neutrophil function covers the well known primary cellular defects such as Chronic Granulomatous Disease, myeloperoxidase deficiency and the Chediak-Higashi syndrome in addition to those abnormalities of function which occur as a consequence of abnormal humoral immunity or in association with systemic disease. There is little direct description of laboratory methodology, although this would be readily available from the literature references; and the rapidly expanding subject of leukapheresis and granulocyte transfusion is hardly touched upon.

The book is well produced, with numerous figures and diagrams. The index is extensive and comprehensive, as befits a reference volume. It will be of use to clinicians in the fields of infectious diseases, immunology, haematology and rheumatology, and an essential addition to the library of experimental biologists, particularly those with an interest in host defence in general and in the neutrophil in particular.

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Soil constituents

The Chemistry of Soil Constituents. Edited by D. J. Greenland and M. H. B. Hayes. Pp. 469. (Wiley: London and Chichester, UK, 1978.) £26.

THIS enterprise is most welcome, as apart from the two encyclopaedic volumes (*Soil Components*, Springer: Berlin, 1975) edited by J. E. Gieseking, no advanced textbook of soil chemistry has been published for many years. The editors consider their subject falls naturally into two parts, the present volume *The Chemistry of Soil Constituents* covering static aspects, and a volume to be published shortly *The Chemistry of Soil Processes* covering dynamic aspects.

The introductory chapter (28 pages) by the editors places the topics of later chapters in the context of soil science as a whole. Chapter 2 (150 pages) entitled 'The Structures and Chemistry of Soil Clay Minerals' by the Rothamsted team of G. Brown, A. C. D. Newman, J. H. Rayner and A. H. Weir is a book in itself. Although clear accounts of the classical aluminosilicate clays already exist, for example, by Grim, this chapter treats the inorganic oxides and fibrous magnesium silicates more fully. Recent developments in two directions are particularly well described. Many soil clays consist of interstratified minerals in which different types of unit—kaolinite, smectite, and so on—alternate in a regular or irregular manner, and the account of the unravelling of these structures is very valuable. Soils developed from weathered volcanic ash commonly have such unusual features as a bulk density less than 0.85 g cm^{-3} . They contain the thread-like mineral imogolite and amorphous allophane; and the confused literature on these substances has been clarified. The excellent electron micrographs are a feature of this chapter. Much trouble has been taken to show complicated structures in two-dimensional diagrams, but this remains a problem, and I would like to have seen some stereopairs.

Chapter 3 (122 pages) entitled 'The Chemistry of Soil Organic Colloids' by M. H. B. Hayes and R. S. Swift deals with the chemical and physical properties of humified substances—not the materials from which they are derived, so lignin and polyphenols, for example, receive little mention. Those who have already decided that humus chemistry is a complex topic best left to its specialist devotees are unlikely to

change their opinion on reading this chapter. The authors rightly emphasise experimental methods of fractionating the humus moieties, and discuss how reliably they have been identified. The size, shape and weight of humus polymers are clearly described. Humic substances are distinguished in this account from "compounds belonging to recognisable classes, such as polysaccharides, polypeptides and altered lignins". The polysaccharides are thoroughly treated in a separate section, but the organic forms of nitrogen, sulphur and phosphorus receive no attention.

The rest of the book concentrates on surface phenomena. C. J. B. Mott and D. J. Greenland (chapter 4, 29 pages) describe the structure of the mineral surfaces and the determination of their area. G. W. Arnold (chapter 5, 44 pages) deals with surface charges and V. C. Farmer (chapter 6, 38 pages) with the structure of water on the surfaces. Together these chapters contain by far the most coherent account of soil surfaces yet assembled. Arnold's discussion of the point of

zero charge in soils is particularly illuminating. I wish he had treated the effect of Ca^{2+} ions in ordering adjacent clay tactoids more thoroughly. The electrical double layer theory he so well describes does not account for this effect, on which the structure of most soils, and thus agriculture, depends. This latter section of the book cries out for a discussion of the affinity of the various surfaces for cations and anions, and a comprehensive account of cation and ligand exchange. These matters are to be treated in the second volume.

The writing throughout is clear and concise, and there are few serious errors. The index is good and the general standard of production high—so too is the price, though the scientific value per page is well above average. The book is unlikely to be replaced for at least a decade, so I rate it a good though expensive buy for all advanced students of soil chemistry.

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Introductory oceanography

Introductory Dynamic Oceanography. By S. Pond and G. L. Pickard. Pp. 240. (Pergamon: Oxford, 1978.) Hardback £15; paperback £5.

ALTHOUGH a number of books published recently have given a general introduction to physical oceanography, a need has remained for a textbook providing a sound dynamical treatment, starting from first principles. This gap has been filled by Pond and Pickard's new book, which has evolved from a course of lectures given for a number of years at the University of British Columbia to students drawn from several disciplines. Its origin is reflected in the detailed explanations of many concepts which are frequently taken for granted in textbooks.

The book starts with a description of the properties of sea water and the basic physical laws relating to movements of water in the sea. There follows a clear explanation of the equations of continuity and motion, including a good discussion of the Coriolis effects. The influence of non-linear terms is given a separate chapter, which includes a discussion of the scales of terms in the equations of motion and introduces the role of the Rossby, Ekman and Richardson numbers in this context.

The chapter devoted to frictionless geostrophic flow gives a thorough treatment of the basic dynamics and its practical application to oceanographic data. The longest chapter in the book is that on currents with friction, which starts with the classical Ekman spiral theory and leads on to solutions applicable to the large scale wind-driven circulation of the oceans. The treatment of the boundary layer approach given in the last part of the chapter is more advanced than the rest but is well done.

A feature of particular interest is the inclusion of a chapter on numerical models. Without going into computational details, it gives a good account of the principles of modelling the ocean circulation and the treatment of boundary conditions, followed by a description and critical discussion of several individual models. The chapters on waves and tides are less detailed than the rest of the book and are largely qualitative. There are, however, other textbooks which deal with these topics.

The authors have succeeded in producing a textbook which will not only give the reader a basic understanding of physical oceanography but also bring him to the stage where he can appreciate advances being made at the frontiers of the subject.

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